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Quality related substrates and enzyme in tea as influenced by weather parameters

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Abstract

Recent threat on global warming and change in climate are not only the topic related with agricultural and biomass productivity; but on the basis of quality consciousness consumers demand, agriculturalists are indebted to offer due importance to the quality of the agricultural commodities. Quality attributes depends mainly upon the biochemical constituents. How far climatic changes influence the quality attributes of tea are unknown. In the pilot scale study, polyphenols, catechins and their oxidative enzyme are considered and variations in their ratios with respect to prevailing climatic conditions of the Anamallais are attempted. Crop shoots were collected at monthly intervals and subjected to determination of polyphenols, catechins and polyphenol oxidase assay. Weather data collected at UPASI meteorological observatory were used for correlation and factor analysis. Irrespective of the clones, "Cambod" cultivars registered higher quantum of polyphenols followed by "Assam" cultivar. Clones studied within the taxonomic group also significantly varied among them, irrespective of the sampling time. As the catechins are polyphenol derivates, the same trend was observed with total catechin content. Among the clones, SA-6 registered least amount of polyphenols and catechins when compared to other clones. Ratio of catechin to polyphenol exhibited different trend; "Cambod" cultivars registered higher values in catechin, polyphenol ratio followed by "China" and "Assam" cultivars. Polyphenol oxidase activity was significantly varied among the jats and the clones within the taxa. Microclimatic variables played an important role in accumulation of predominant quality constituents, polyphenols or catechins and the enzyme, poly phenol oxidase. Sunshine hours positively related with the above said biochemicals while rainfall has negative influence on the biochemical constituents. Maximum temperature had positive and significant correlation with quality constituents whereas, minimum temperature registered negative impact on their production. Relative humidity recorded at 8.00 am and 2.30 pm exerted negative influence on polyphenols, catechins and PPO activity. Multiple regression models derived based on the climatic variables are presented and discussed in detail.

Keywords: Climatic variables, prediction model, quality constituents, tea

Introduction

Quality of commercial made tea is brought about by complex biochemical constituents including the substrates, enzymes and their interactions. Even though the raw materials of the harvested crop and the manufacturing methodologies influence to an extent upon the quality and falavour profile of the final tea product, substrate/enzyme specificity is an important factor in black tea manufacture (Marimuthu and Raj Kumar, 2001). It has been established that the quality related enzymes and substrates are influenced by several factors like cultivar type, maturity of the harvested crop and seasons besides the agricultural operations carried out in the plantations (Marimuthu, 2000; Raj Kumar, 2005). Tea growing regions of south India, being located along the Western Ghats are not adversely affected by the gradual change in climatic conditions in terms of productivity and the cropping pattern (Raj Kumar and Mohan Kumar, 2009; 2010). But how far the patterns of weather parameters instrumental to affect the quality attribute of tea is unknown. Recent threat on global warming and change in climate are not only the topic related with agricultural and biomass productivity; but as a demand of the quality consciousness consumer, agriculturalists are indebted to offer due importance to the quality produce. Polyphenols, catechins and their oxidative enzymes are the important components of quality (Roberts and Fernando, 1981; Thanaraj and Seshadri, 1990; Ramakrishnan *et al*., 2002) and hence variations in their composition with respect to changing weather parameters are attempted in the present study.

Materials and Methods

Experimental

The experiments were conducted at UPASI Tea Research Institute during 2009. Three tea clones each representing "Assam" (UPASI-9, UPASI-16 and UPASI-18), "China" (UPASI-10, UPASI-15 and SA-6) and "Cambod" (UPASI-8, UPASI-17 and CR-6017) cultivars growing under uniform cultural conditions were selected for the study (Raj Kumar, 2005). All cultural practices were carried out as per UPASI guidelines, uniformly irrespective of the cultivars/clones (Muraleedharan *et al*., 2007).

Sampling

Crop shoots comprising three leaves and a bud from the above said tea clones were collected individually, at monthly intervals during the study period. Minimum three individual crop shoot samples were collected at random from the selected mother bushes for biochemical analyses. Weather data collected at UPASI meteorological observatory for the study period were used for correlation studies and to compute the regression models. It may be noted that the crop shoot sampling was done at monthly intervals, particularly last week of each month, mean monthly weather parameters were used as such for statistical analysis, since the sampling was done at end of each month.

Quantification of polyphenol oxidase, polyphenol and catechin

Crop shoots were subjected to biochemical analysis, particularly, polyphenols, catechins and polyphenol oxidase (PPO). Although, three leaves and buds are collected from the field, PPO estimation was done with two leaves and a bud following the method described by Singh and Ravindranath (1994) and these were expressed as U/mg protein. Protein was estimated following the method of Bradford (1976). Quantification of catechin was done following the methodology of Swain and Hillis (1959) while polyphenols was determined adopting the methodology of Dev Choudhury and Goswami (1983) and both catechins and polyphenols were expressed as percent dry weight. Ratios of catechins to polyphenol, polyphenol to polyphenol oxidase and catechins to polyphenol oxidase were also computed.

Statistical analysis

Factorial design statistical analysis was adopted in the present study where three factors cultivar type, sampling time (month) and clones were considered for primary, secondary and tertiary variables (Gomez and Gomez, 1984). Mean monthly climatic variables were correlated with the representative values of substrates/ enzymes and their ratios. Factor analysis was carried out with SPSS Ver. 10.0 software.

Results and Discussion

When considering the quality of tea, biochemical constituents that play a paramount role are polyphenols and their oxidative enzymes. As mentioned earlier, endogenous level of oxidative enzyme, PPO which acts upon polyphenols and catechins, prime importance was given to these enzyme and substrates (Ramakrishanan *et al.*, 2002). Tea contains about 30 % of dry matter as polyphenols out of which two parts is comprised of catechins and remaining portion accounts for other phenol derivatives. The enzyme PPO acts upon the polyphenols during processing of green leaves which in turn contributes the black tea pigments theaflavins and thearubigins.

Irrespective of the clones, jats showed a distinct variation in polyphenol content where "Cambod" cultivars registered higher quantum of polyphenols followed by "Assam" cultivar while "China" hybrids recorded significantly lower amount of polyphenols (Table 1). Clones studied within the taxonomic group also significantly varied among themselves, irrespective of the sampling time (months). Mean monthly polyphenol ranged between 26.69 and 31.28 %, irrespective of the clones/cultivars and statistically significant in accordance with prevailing climatic conditions.

As the catechins are polyphenol derivates, the same trend was observed with total catechin content with respect to the clones jats and sampling period (Table 2). Among the clones, SA-6 registered the least amount of polyphenols and catechins (27.08 and 16.64, respectively) when compared with other clones. It has been noticed that the interaction between the jat Vs clone and jat Vs sampling periods are statistically significant in certain occasions.

Ratio of catechin to polyphenol exhibited different trend; "Cambod" cultivars registering higher values in catechin, polyphenol ratio followed by "China" and "Assam" cultivars. Among the clones, SA-6 recorded significantly lower values of catechin to polyphenol ratio. Interestingly, being an estate selection recognised for its quality, CR-6017 registered the values on par with SA-6 in terms of catechin to polyphenol ratio, irrespective of the sampling time (months). Catechin to polyphenol ratio ranged from 0.577:1 to 0.693:1 during different months and irrespective of the clones.

Table 1. Seasonal and cultivar variation in polyphenol content in crop shoots of various tea clones

						Polyphenol (%)							
Month	**UP-9**	**Assam UP-16**	**UP-18**	**Mean (Jat x month)**	**UP-10**	**China UP-15**	**SA-6**	**Mean (Jat x month)**	**UP-8**	**Cambod UP-17**	**CR**	**Mean (Jat x month)**	**Mean (month)**
Jan.	30.20	28.56	31.26	30.01	31.54	31.61	29.78	30.98	30.98	29.69	30.42	30.36	30.45
Feb.	30.27	26.96	30.99	29.41	32.24	31.92	26.56	30.24	31.18	30.91	31.01	31.03	30.23
Mar.	29.86	33.10	31.30	31.42	29.88	30.86	27.24	29.33	29.80	34.15	32.58	32.18	30.97
Apr.	31.73	33.26	30.61	31.87	30.32	28.57	28.64	29.18	31.39	33.88	32.96	32.74	31.26
May	30.19	28.84	29.49	29.51	30.55	27.85	27.17	28.52	29.73	31.34	30.75	30.61	29.55
June	30.86	28.55	29.08	29.50	29.91	27.32	27.16	28.13	29.32	28.90	28.41	28.88	28.83
July	29.14	26.56	27.88	27.86	27.51	27.52	24.53	26.52	29.17	26.76	28.26	28.06	27.48
Aug.	28.69	27.35	28.26	28.10	26.04	27.17	22.09	25.10	28.68	26.64	25.29	26.87	26.69
Sep.	30.70	30.26	28.75	29.90	27.14	29.98	28.04	28.39	27.65	33.09	31.46	30.73	29.67
Oct.	32.28	28.36	32.28	30.97	31.19	31.67	28.15	30.34	30.45	28.05	29.45	29.32	30.21
Nov.	31.89	32.10	30.96	31.65	29.76	33.05	26.97	29.93	33.57	33.65	29.55	32.26	31.28
Dec.	30.13	30.15	31.61	30.63	30.86	31.30	28.57	30.24	30.78	28.46	31.28	30.17	30.35
Mean: Clone	30.50	29.50	30.21		29.75	29.90	27.08		30.23	30.46	30.12		
Mean: Jat				30.07				28.91				30.27	

Statistical significance:		S.E.	C.D.	C.V. (%)
	Jat	0.06	0.12	5.08
	Clone	0.06	0.12	
	Jat x month	0.71	1.40	
	Month	0.24	0.47	
	Jat x Clone	0.18	0.35	

Table 2. Seasonal and cultivar variation in catechins in crop shoots of various tea clones

						Polyphenol (%)							
Month	**UP-9**	**Assam UP-16**	**UP-18**	**Mean (Jat x month)**	**UP-10**	**China UP-15**	**SA-6**	**Mean (Jat x month)**	**UP-8**	**Cambod UP-17**	**CR**	**Mean (Jat x month)**	**Mean (month)**
Jan.	19.71	18.55	21.36	19.87	23.06	21.95	19.40	21.47	20.81	21.86	18.26	20.31	20.55
Feb.	20.89	17.55	20.63	19.69	23.58	22.87	17.51	21.32	21.61	23.00	21.15	21.92	20.98
Mar.	20.90	21.38	19.68	20.65	20.36	20.59	15.60	18.85	22.62	24.02	21.70	22.78	20.76
Apr.	20.53	22.73	19.77	21.01	20.35	21.22	17.45	19.67	23.73	24.72	22.82	23.76	21.48
May	20.60	19.64	19.98	20.07	20.38	21.07	17.29	19.58	21.49	23.32	20.31	21.71	20.45
June	18.66	16.55	18.61	17.94	19.06	16.29	14.98	16.78	16.53	18.35	18.43	17.77	17.50
July	16.88	14.16	17.75	16.26	16.19	16.68	13.67	15.51	17.46	14.57	15.60	15.88	15.88
Aug.	17.02	15.25	18.22	16.83	16.63	17.08	14.22	15.98	18.38	14.49	15.00	15.96	16.25
Sep.	16.69	17.68	16.74	17.04	16.97	16.59	16.67	16.74	16.87	19.14	20.28	18.76	17.51
Oct.	20.48	18.70	22.18	20.45	20.36	20.70	17.68	19.58	21.64	18.53	15.35	18.51	19.51
Nov.	20.12	19.66	17.70	19.16	17.72	19.08	17.03	17.94	20.01	20.93	17.40	19.45	18.85
Dec.	18.54	19.54	19.71	19.26	22.53	21.03	18.16	20.57	20.00	20.72	19.00	19.91	19.91
Mean (Cl)	19.25	18.45	19.36		19.77	19.60	16.64		20.10	20.30	18.78		
Mean (jat)				19.02				18.67				19.73	

Statistical significance:		S.E.	C.D.	C.V. (%)
	Jat	0.05	0.11	7.21
	Clone	0.05	0.11	
	Jat x month	0.65	1.28	
	Month	0.21	0.43	
	Jat x Clone	0.16	0.32	

Polyphenol oxidase activity was significantly varied among the jats (819.76, 539.89 and 377.06 U/mg protein registered by "Assam", "Cambod" and "China" jats, respectively) and the clones within the taxa (Table 3). Considering the seasons (range irrespective of the clones/cultivars, 432.70 to 652.56 U/mg proteins), though there was significant variation in PPO, the variation was not very wide as observed among the jats/clones. Ratios of polyphenols to PPO and catechins to PPO also exhibited variations.

Climatic variables played an important role in synthesis and accumulation of predominant quality

Table 3. Seasonal and cultivar variations in polyphenol oxidase activity in tea clones

Month	Polyphenol (%) UP-9	Assam UP-16	UP-18	Mean (Jat x month)	UP-10	China UP-15	SA-6	Mean (Jat x month)	UP-8	Cambod UP-17	CR	Mean (Jat x month)	Mean (month)
Jan.	724.50	1091.00	1112.21	975.90	334.24	437.50	309.50	360.41	809.50	441.54	438.50	563.18	633.17
Feb.	706.25	1148.23	1128.23	994.24	325.24	421.85	296.23	347.77	821.36	428.54	423.36	557.75	633.25
Mar.	736.23	1201.12	1028.53	988.63	348.54	436.25	302.25	362.35	790.28	452.36	482.78	575.14	642.04
Apr.	692.23	1156.25	1154.23	1000.90	375.92	389.81	346.90	370.88	864.29	403.25	490.18	585.91	652.56
May	408.15	962.79	890.00	753.65	289.90	359.12	284.66	311.23	673.22	366.82	456.44	498.83	521.23
June	408.99	743.29	766.88	639.72	276.11	324.76	267.33	289.40	630.23	320.13	453.06	467.81	465.64
July	716.58	825.64	704.69	748.97	529.24	464.93	422.39	472.19	515.39	500.31	689.77	568.49	596.55
Aug.	625.85	897.21	568.10	697.05	348.67	487.68	353.99	396.78	494.79	500.31	503.12	499.41	531.08
Sep.	673.59	613.24	338.53	541.79	364.42	306.65	285.58	318.88	441.77	448.38	422.12	437.42	432.70
Oct.	751.00	876.32	689.23	772.18	540.23	489.36	321.65	450.41	524.36	531.25	656.23	570.61	597.74
Nov.	756.21	863.25	679.53	766.33	521.36	496.23	395.26	470.95	531.25	533.28	691.23	585.25	607.51
Dec.	743.11	1034.28	1096.01	957.80	343.09	454.28	323.14	373.50	798.21	454.23	454.16	568.87	633.39
Mean (Cl)	661.89	951.05	846.35		383.08	422.37	325.74		657.89	448.37	513.41		
Mean (jat)				819.76				377.06				539.89	

Statistical significance:		S.E.	C.D.	C.V. (%)
	Jat	4.73	9.27	20.80
	Clone	4.73	9.27	
	Jat x month	56.77	111.26	
	Month	18.92	37.09	
	Jat x Clone	14.19	27.82	

constituents, polyphenols/catechins and their oxidative enzyme, PPO (Table 4). Sunshine hours positively related with the polyphenols, catechins and PPO while rainfall exhibited negative impact on the above biochemical constituents (Table 5). Maximum temperature had positive and significant correlation with quality constituents whereas, minimum temperature registered negative impact on their production. Relative humidity recorded at 8.00 am and 2.30 pm exerted negative influence on polyphenols, catechins and PPO activity. Multiple regression models developed in the present study could be used to predict the expected quality related enzyme and substrates during different periods under the prevailing conditions of the Anamallais.

The enzyme and substrates (polyphnol and catechins) could be predicted using weather parameters. Confidence levels are 98.6 % against polyphenols, 98.1 % against catechins while it was 75.0 % against the PPO. Formula for prediction of polyphenols using the climatic variables is: polyphenols = (0.334) + sunshine hours (-0.181) + rainfall in cm (0.0284) + max. temperature (1.252) + min. temperature (-0.284) + RH maximum (-0. 0517) + RH minimum (0. 0704). Correlation coefficient value (0.986) is highly significant at 1 % probability. Among the independent variables, sunshine hours, minimum temperature and RH maximum had negative relationship with prediction of polyphenols.

Table 4. Mean monthly variations in weather parameters and biochemical constituents

	Temperature (°C) Mean min.	Mean max.	Relative Humidity (%) 8.30 a.m	2.30 p.m	Rain fall (mm)	Rainy days (no.)	Mean sunshine period (h/day)	Sunny days (no.)	Poly phenols (%)	Catechins (%)	PPO(U/mg protein)
Jan.	11.6	27.8	79	54	0	0	8.4	31	30.36	20.31	563.18
Feb.	13.9	29.9	76	43	0.8	0	9.6	28	31.03	21.92	557.75
Mar.	16.1	29.9	83	65	190.8	8	6.5	31	32.18	22.78	575.14
Apr.	15.7	29.6	87	69	32.2	2	5.5	27	32.74	23.76	585.91
May	17.8	27.6	89	79	263.8	10	4.1	28	30.61	21.71	498.83
Jun.	18.3	25.2	93	83	455.6	23	2.4	18	28.88	17.77	467.81
Jul.	19.1	22.7	94	90	1223.6	27	0.5	7	28.06	15.88	568.49
Aug.	19.0	24.1	94	86	379.8	23	1.4	18	26.87	15.96	499.41
Sep.	19.1	24.6	92	86	639	19	2.4	19	30.73	18.76	437.42
Oct.	18.1	26.1	89	82	371	13	5.3	28	29.32	18.51	570.61
Nov.	17.1	26.5	87	78	164.6	10	4.5	28	32.26	19.45	585.25
Dec.	14.7	27.2	87	79	73	8	5.6	29	30.17	19.91	568.87

Table 5. Correlation coefficient values and interaction between biochemical constituents and seasons

	Sunshine hours	Rainfall cm (total)	Temperature (°C) Max.	Temperature (°C) Min.	Relative humidity (%) Max.	Relative humidity (%) Min.	Polyphenols (%)	Catechins (%)	PPO (U/mg protein)
Sunshine h	1.000								
Rainfall	-0.802	1.000							
Max.temp.	0.875	-0.839	1.000						
Min.temp	-0.889	0.729	-0.722	1.000					
RH max.	-0.969	0.727	-0.821	0.895	1.000				
RH min.	-0.918	0.677	-0.810	0.850	0.964	1.000			
Polyphenols	0.715	-0.630	0.917	-0.599	-0.654	-0.636	1.000		
Catechins	0.729	-0.712	0.946	-0.584	-0.674	-0.661	0.973	1.000	
PPO	0.673	-0.604	0.648	-0.620	-0.604	-0.515	0.601	0.531	1.000

Values > 0.602 & 0.735 are statistically significant at 5 and 1 per cent, respectively

Similarly catechins could be predicted with the regression model: catechins = (-11.282) + sunshine hours (-0.516) + rainfall in cm (0.0195) + max. temperature (1.726) + min. temperature (-0.153) + RH maximum (-0. 193) + RH minimum (0. 0823). Correlation coefficient value, 0.981 is highly significant at 1 % level.

Formula for prediction of PPO is: PPO = (271.661) + sunshine hours (12.396) + rainfall in cm (0.196) + max. temperature (10.392) + min. temperature (-7.445) + RH maximum (-2.262) + RH minimum (3.274); r = 0.750. Even though the confidence level is relatively lesser than the substrates, the values are significant at 1 % probability. Unlike the substrates, sunshine hours and RH minimum exhibited positive relationship with PPO.

Ecological variables are independent in nature which influenced the biochemical constituents of tea to a great extent. Out of eight climatic variables, number of rainy and sunny days was excluded from the factor analysis. Sunshine hours accounted for 73.37 % variation followed by rainfall (13.81 %). Cumulative effect of these two variables contributed 87.19 %. Considerable amount of variation was contributed by both maximum and minimum temperatures (~10 %), while other variables like relative humidity collectively showed negligible impact.

Higher amount of polyphenols and catechins observed during post monsoon/winter and summer periods are attributed to the longer period of sunshine hours which in turn triggers the biosynthesis of phenolic compounds (Yao *et al.*, 2005). Fermenting ability of harvested crop shoots solely depend on the endogenous level of enzymes (Marimuthu, 2000), particularly the PPO which initiates the process. Earlier Marimuthu (2000) reported that the collective response of oxidative enzymes gained importance and offered a better index to identify certain quality parameters. Irrespective of the cultivars, it can be noticed that the tea leaves harvested during summer recorded higher amount of enzyme which are responsible for transformation of phenolic compounds in to desired quality attributes (Robertson, 1992). Variations in enzyme activities are due to longer photoperiod and concurrent enhancement in metabolic activities. Lower enzyme activity during monsoon was observed in the present study but Pruidze *et al.* (2003) reported higher levels of PPO. Positive and significant correlation existed between maximum temperature and sun shine hours favoured the accumulation of polyphenols and catechins as mentioned earlier (Yao *et al.*, 2005).

From the data presented, biochemical constituents related to quality attributes are influenced by weather parameters. Sunshine hours showed a positive correlation with the synthesis and accumulation of polyphenols, catechins and PPO under prevailing conditions of the Anamallais. It may be noted that the temperature (max) ranges between 22.66 and 29.73 °C where it exerted positive impact on above quality related biochemical constituents; but the temperature rises beyond 30 °C, the fate of these biomolecules are not yet brought out by any report even under *in vitro* or *in situ* conditions. However, rise in temperature had a quadratic relationship with atmospheric carbon dioxide assimilation of tea leaves (Raj Kumar, 2003). This type of relation could be expected when the atmospheric temperature rose from the existing scenario. Minimum temperature had a weak negative relationship with substrates but it influence on the enzyme was very strong, where the correlation coefficient values are significant at 1 % level. Relative humidity recorded at 8.30 am and 2.30 pm negatively related with all quality related substrates and enzymes documented in the present study. This is due to the existing relationship among the climatic variables.

Earlier regression models were developed to predict the quality attributes of made tea using the green leaf constituents (Joseph Lopez, 2002; Ramakrishnan *et al.*, 2002). Clonal and seasonal variations were also reported from time to time (Gulati and Ravindranath, 1996; Gulati *et al*., 1999). But the results are not subjected to the factor analysis to fix the contributory factor. As far our knowledge is concerned this is the pioneering report where certain biochemical constituents are subjected to factor analysis along with climatic variables. Results of the factor analyses denoted that sunshine hours is the primary contributing factor on quality related enzyme and substrates. Regression models developed herein may be applicable to the Anamallais only because of the data pertaining to this region is exploited to derive the regression model. Tea clones representing all the cultivated *Camellia* hybrids only were considered in the present study. Application of the regression model to predict the substrates/enzymes using the weather parameters variables pertaining to seedlings may deviate to an extent. However, for confirmation, the study needs to be further experimentation with seedlings as well.

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