ORIGINAL RESEARCH REPORT:

EFFECT OF LEVEL OF CHEMICAL WITHER AND DURATION OF FERMENTATION ON BIOCHEMICAL COMPOSITION OF MADE TEA

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ABSTRACT

A series of withering and fermentation experiments in black tea processing were conducted in Environmentally Controlled Manufacturing (ECM) system. Three different hygrometric conditions (i.e. T_1, T_2 & T_3) during withering and five different fermenting times during fermentation were maintained to assess the variation in quality components of black tea. To prepare the samples fresh tea leaves of clones TV1 and TV26 were collected from the experimental garden of Tea Research Association, Jorhat. Composition of five major catechins i.e. EGC, +C, EC, EGCG and ECG in fresh (F) as well as in withered (W) leaf and theaflavins (TF), thearubigins (TR) and its fractions, total color (TC), brightness (B) and volatile flavor components (VFC) in black tea were estimated using High Performance Liquid Chromatography (HPLC), Gas Liquid Chromatography (GLC) and UV/Vis spectrophotometer. In this set of experiment total withering time was maintained 12hrs, where the moisture loss in the initial hours was restricted by manipulating the wet bulb depression (WBD). Under the least WBD (T_3) the chemical changes (chemical withering) was most favorable from quality point of view. Fermentation time 50min was found better for obtaining brighter tea in TV1 whereas for TV26 it was 70min. It was observed that Flavour Index (FI) in clone TV1 was higher in treatment T_3 while for clone TV26 it was T_1

Keywords: *Camellia sinensis*, Tea, catechins, theaflavins (TF), thearubigins (TR), VFC, ECM, manufacturing variation, hygrometric condition**,** chemical wither, physical wither

1. INTRODUCTION

Black tea is one of the most popular and extensively consumed beverage worldwide. Besides the medicinal value people like tea for its aroma and stimulating effect. Quality of tea is an integrated perception of taste and aroma. As tea processing is a complex biochemical phenomenon where four different processing stages are involved, therefore, critical study of the stages are essential to understand the biochemical changes and their role in quality of black tea. The processing stages are withering, rolling, fermentation and drying. Withering or partial desiccation allows certain chemical changes to occur and a reduction of leaf moisture content from 78-80 % by weight down to 68-70% depending on the type of tea to be manufactured. Development of taste and aroma are dependent on different process variables during withering. Moisture content of leaf is one of the most limiting factors for the progressive

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cell damage during tea processing (Selvendran and King,1976). Normal withering involves loss of moisture along with simultaneous occurrence of some biochemical changes to form certain quality attributes. Chemical withering is important to enhance biochemical components, which contribute to quality of black tea (Bhatia, 1962; Sanderson 1964).

Polyphenols or catechins, the major components of green leaf, convert to two important classes of pigments known as theaflavins (TF) and thearubigins (TR), which are formed during fermentation. TF and low molecular TR (TR1), which have astringent properties, contribute a lot to quality of tea. Therefore, the study of the formation of these biochemical constituents, which contribute towards the brightness, briskness, depth of colour and mouth feel of the liquor is very important.

Volatile flavoury components are an another important area of study where several macromolecules, enzymes are responsible for production of different VFC. Degradation of macromolecules like carbohydrates and carotenoids increases soluble sugars and formation of terpenoid flavoury compounds during withering (Sanderson and Perera , 1965; Takeo and Tsushida,1980; Hazarika and Mahanta 1983). Dehydration of leaf during withering accelerates the activity of lipoxygenase enzyme, which is responsible for degradation of unsaturated fatty acids to C_6 aldehydes and alcohols (Bhuyan and Mahanta, 1989; Selvendran and others 1978; Wright and Fishwick 1979). All the complex biochemical reactions during processing is dependent on various physical parameters and their precise control is very essential for optimal formation of biochemical quality components (Tamuly and others 2005). It was reported that withering has great impact on the production of aroma and flavour compounds and is very essential stage to improve the black tea quality (Saijo and Takeo 1973; Saijo 1977; Takeo and Mahanta 1983a and 1983b; Fernando and Roberts 1984; Mahanta and Baruah 1989; Devchoudhury and Bajaj 1980; Takeo 1984; Hatanaka and others 1987). The increase in amino acids concentration has effect on quality of tea as they undergo degradation during processing to give some volatile aldehydic compounds (Saijo 1973; Sanderson and Graham 1973; Motoda 1979), for example 2-methyl propanal, 2-methylbutanal, pentanal and phenyl acetaldehyde are formed from valine, leucine, isoleucine and phenylalanine respectively. Generation of floral tea aroma is a result of stresses given to juvenile tea leaves in withering. Before fermentation leaves should be subjected not only to withering but also slight injury to leaves is essential for generation of floral aroma (Sakata K and others 2004). It was reported that alcoholic aroma compounds having floral fruity aroma have major contribution to quality of black tea. These compounds are present as glycosides in tea leaves and are released by endogenous enzymes glycosidase during withering, rolling and fermentation.

The objective of this study was to enhance the essential biochemical parameters for upgradation of quality of tea by modifying the withering and fermentation conditions.

Material and Methods

Sample collection Tea shoots of cultivars TV-1 and TV-26 were collected from the experimental garden of Tocklai Experimental Station, Jorhat, where 7 days plucking round was maintained.

Black tea processing The processing experiments were carried out in ECM system procured from Tea Craft, UK where it was possible to attain the targeted temperature and humidity, precisely during withering and fermentation (Nigel and Richard, 1991,1993)

Withering conditions Tea shoots (moisture, 79% ±1) were withered for a period of 12 hrs where initial 5 hrs leaves were allowed to wither in three different hygrometric conditions, T_1, T_2 and T_3. 69.5-70.2% percent was the final moisture content for all the treatments after 12hrs of withering which was achieved in remaining 7 hrs. Details of T_1, T_2 and T_3 are given below:

T_1: Leaves were allowed to undergo wither for 5hrs in 2° wet bulb depression (WBD), where dry bulb temperature was maintained at 30° C.

Leaf moisture after initial 5hr stress was 74.8% ±1

T_2: Leaves were allowed to undergo wither for 5hrs in 1° wet bulb depression (WBD), where dry bulb temperature maintained at 30° C.

Leaf moisture after initial 5hr stress was 76.3% ±1

T_3: Leaves were allowed to undergo wither for 5hrs in 0° wet bulb depression (WBD), where dry bulb temperature was maintained at 30° C.

Leaf Mosisture after initial 5hr stress was 77.8% ±1

Rotorvan CTC Withered samples were put in mini rotorvan (size, 18"x 3") followed by 3 cut using 6" eight TPI crush, tear and curl (CTC) machine.

Fermentation Three cut CTC samples were allowed to ferment for different duration i.e 40min, 50min, 60min, 70min and 80min. The temperature and humidity of entire fermentation period were maintained at 30°C and 90% respectively in ECM and samples were spread in trays with a thickness of 2cm (Nigel,1995, 2001)

Drying Leaves were dried in mini fluid bed dryer (FBD), supplied by Tea Craft with inlet temperature110^{0}C for about 20 min till the moisture content reduced to about 3 – 4 %. FBD can precisely control the inlet temperature and air flow. Dryer mouth samples were used as black tea for chemical analysis.

ANALYTICAL METHODS

TF and TR were estimated using standard methods (Ullah and others 1984; Annual Report TRFK, 1996).

Extraction, identification and quantification of volatile flavor constituents

The aroma concentrate was prepared from 100g of black tea by steam distillation under reduced pressure for 45min using the method reported earlier (Takeo and Mahanta, 1983a).

The concentrate thus obtained was analyzed in Varian GC model CP 3800 equipped with flame ionization detector (FID) and 60m x 0.25mm CP-wax 52CB column. Both the injector and detector temperatures were kept at 250^{0}C. The column oven temperature was maintained from 50^{0}C to 200^{0}C at the rate of 2^{0}C/min and total run time was 105 min. Peaks were identified by comparing with authentic compounds from Sigma Chemicals. The amount of each component was determined and expressed as the ratio of each peak area to that of internal standard in the chromatogram(Hazarika M and others 2005).

Analysis of catechins was estimated by HPLC using phenyl-hexyl column as per the standard ISO method (ISO/CD 14502-2). Catechins standards were obtained from Sigma Chemicals.

Experimental design and statistical analysis

All the trials were conducted under laboratory conditions and were statistically designed. Randomized Block Design (RBD) was adopted for all trials with four replications. Withering conditions and fermentation time as given in the methodology were considered as treatments. Data generated from the trials were statistically analyzed to ascertain significance between treatments. Statistical analysis was done using ANOVA (Analysis of Variance) technique.

RESULTS:

Catechin contents of fresh and withered leaf in three different hygrometric conditions of clone TV1 and TV26 are presented in Table 1a and 1b. In this study it was found that restriction of moisture loss helps to prevent degradation/loss of catechins in the withered leaf. It was observed that degradation of EGCG was minimum in T_3 condition where moisture loss is minimum. Degradation of total catechin was also minimum under 100%(T_3) humid condition followed by 93%(T_2) and 86%(T_1) in both the clones TV1 and TV26 as shown in Figure1.

Table 1a. Effect of Wet Bulb Depression during chemical withering on catechin (% Dry wt.) degradation in TV1.

Treatment	EGC		+C		EC		EGCG		ECG		TOTAL	
	F	W	F	W	F	W	F	W	F	W	F	W
T1		1.47		0.47		1.05		6.01		4.03		13.10
T2	1.82	1.85	0.93	0.49	1.15	1.07	7.10	6.02	4.30	3.93	15.30	13.36
T3		1.72		0.64		1.00		6.17		3.98		13.50
F values treatment		0.85 N.S		0.19 N.S		0.04 N.S		0.14 N.S		0.06 N.S		0.10 N.S

Table 1b. Effect of Wet Bulb Depression during chemical withering on catechin (% Dry wt.) degradation in TV26

atment t	EGC		+C		EC		EGCG		ECG		TOTAL	
	F	W	F	W	F	W	F	W	F	W	F	W
		3.21		0.18		1.01		9.40		3.70		17.50
	3.30	2.87	0.73	0.40	0.47	0.41	10.12	9.81	4.70	4.11	19.31	17.59
		2.81		0.70		0.76		10.06		3.92		18.25
alues atment		0.50 N.S		3.76 N.S		12.69 **		0.31 N.S		0.95 N.S		0.54 N.S

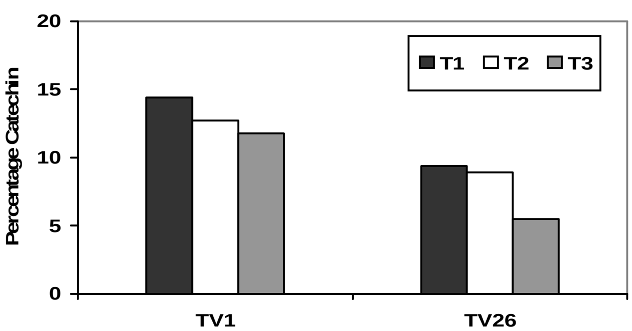

Fig.1 Effect of wet bulb depression on total catechin degradation

Theaflavins (TF), thearubigins (TR), low molecular thearubigin fraction (TRI), total colour (TC) and brightness of black tea are presented in Table 2a and 2b. A significant reduction in TF, TR(I) and brightness was observed in T_1 condition for both the clones TV1 and TV26. Black tea obtained from T_3 shows the highest value of these parameters. Black tea processed at 50min fermentation time for clone TV1 and 70min for TV26 has the highest brightness and briskness. Statistically significant variations in TF, TR(I) and brightness were observed among the treatments in both the clones.

Table 2a. Effect of Wet Bulb Depression on quality parameters in TV1

Treatment	Fermentation time	%TF	%TR	%TR(I)	%TR(II)	%TC	%B
T_1	40min	1.41	20.66	6.15	14.20	7.00	9.80
	50min	1.96	21.91	6.72	14.06	6.97	11.16
	60min	1.80	22.82	6.31	14.55	7.06	10.10
	70min	1.32	23.67	6.22	16.00	7.26	10.12
	80min	0.94	23.78	6.25	16.16	7.11	9.64
T_2	40min	1.89	21.42	6.67	13.04	6.56	14.21
	50min	2.09	21.08	7.87	13.14	6.46	14.78
	60min	1.91	21.33	7.08	13.08	6.48	14.34
	70min	1.52	22.41	7.12	13.77	6.36	12.97
	80min	1.00	22.80	6.75	13.67	6.08	13.40
T_3	40min	1.96	21.08	8.64	14.16	6.67	14.85
	50min	2.32	21.64	9.29	13.88	6.50	16.88
	60min	2.00	21.37	9.01	14.02	6.56	15.21
	70min	1.42	22.00	8.75	13.73	5.80	15.00
	80min	0.98	22.78	8.70	12.86	6.16	14.55
	F values (treatment)	29.04***	0.73NS	7.55**	1.81NS	4.70*	5.98*

Table 2b. Effect of Wet Bulb Depression on quality parameters in TV26.

Treatment	Fermentation time	%TF	%TR	%TR(I)	%TR(II)	%TC	%B
T_1	40min	1.04	20.46	4.28	13.38	6.00	7.36
	50min	1.48	22.14	4.80	13.77	6.93	8.92
	60min	1.93	22.70	4.96	14.35	7.06	9.88
	70min	2.29	23.10	5.38	14.62	7.22	10.86
	80min	1.95	22.73	5.08	14.30	6.97	10.16
T_2	40min	1.00	20.00	4.44	12.14	6.06	12.40
	50min	1.47	21.36	5.00	13.04	6.29	14.15
	60min	1.94	22.08	5.27	13.59	6.82	15.37
	70min	2.39	21.81	6.20	12.75	6.94	17.14
	80min	2.10	22.37	5.59	13.54	6.91	15.70
T_3	40min	0.97	18.67	5.38	12.42	5.13	13.19
	50min	1.56	19.34	7.00	12.90	5.36	14.73
	60min	1.94	20.58	7.87	13.55	6.17	16.82
	70min	2.48	20.15	8.82	13.16	6.23	18.36
	80min	1.99	20.63	8.16	13.46	6.21	17.08
	F values (treatment)	27.72***	1.12NS	17.39***	0.72NS	1.17NS	28.42***

In this investigation we have undertaken a study to observe the changes in VFC profile of made tea for both the clones. Amount of 1-pentanol, trans-2-pentenal, cis-3-hexene-1-ol, nonylaldehyde, linalooloxide–II, linalool, benzaldehyde were estimated less in T_1 while the formation of hexanal,1-pentanol, linalool,1-nonanol, phenylacetaldehyde, terpineol, geraniol, α-ionone, benzylalcohol, 2-phenylethanol and β-ionone were obtained higher in T_3 for clone TV1 (Fig.2.1-2.2).

1-pentanol, trans-2-pentenal, cis-3-hexene-1-ol, nonylaldehyde and linalool were in lower amount in T_1 (Fig.2.3), whereas hexanal, trans-2-hexenal, linalool-oxide-I, benzaldehyde,1-nonanol, terpeniol, nerol, geraniol, α ionone, benzylalcohol and β ionone were found higher in T_1 (Fig. 2.3-2.4) for clone TV26.

It is apparent from the Fig.3 that FI was maximum in T_3 and T_1 for clone TV1 and TV26 respectively. Trans-2-hexenal, with grassy tone in tea flavour was obtained in the highest amount in T_1 (2° WBD) and linalool was the maximum in T_3 treatment for both the clones (Fig.2.1 & 2.3). Optimal condition for the formation of linalool oxide-II for both the clones responded differentially. Linalool oxide- II was generated in the lowest amount in T_1 for clone TV1 and in T_3 for clone TV26. Similarly generation of α-ionone, β-ionone, benzylalcohol etc. were varied in different conditions.

Chemical withering at 0° WBD was favorable for formation of groupII VFC in TV1 in higher proportion resulting superior FI contrary to TV26 where formation of groupI VFC was higher resulting inferior FI (Fig.3). Some essential VFC like geraniol, linalool oxide-II, α-ionone etc. were found lower at T_3 in TV26 causing lower value of FI. So it was apparent that initial moisture loss during withering appears critical for flavour of black tea.

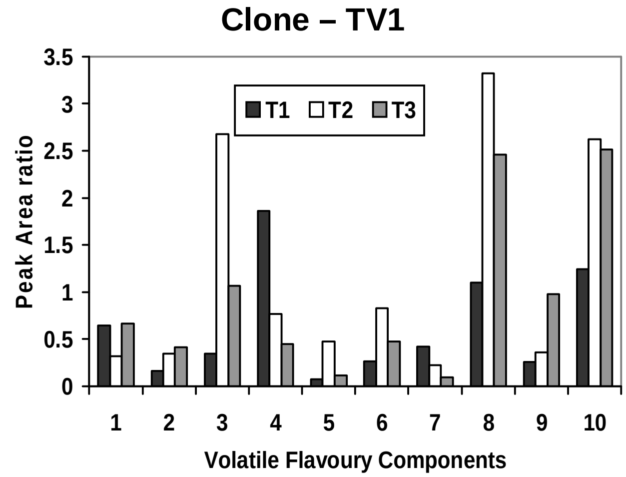

Fig. 2. 1. 1. Hexanal 2. 1-pentanol 3. trans-2-pentenal 4.trans-2-hexenal 5. cis-3-hexen–1-ol 6. Nonylaldehyde 7. Linalool oxide-I 8. Linalool oxide-II 9. Linalool 10. Benzaldehyde.

F* value for** 1. Hexanal, 3.87NS; 2. 1-pentanol, 17.28** 3. trans-2-pentenal 18.80** 4. trans-2-hexenal,11.39** 5. cis-3-hexen-1-ol,117.56
6. Nonylaldehyde, 24.89** 7. Linalool oxide-I, 240.24*** 8. Linalool oxide-II,10.52* 9. Linalool, 10.05* 10. Benzaldehyde, 81.26***
NS: NOT SIGNIFICANT, * SIGNIFICANT AT 5%, ** SIGNIFICANT AT 1%, ***SIGNIFICANT AT 0.1%

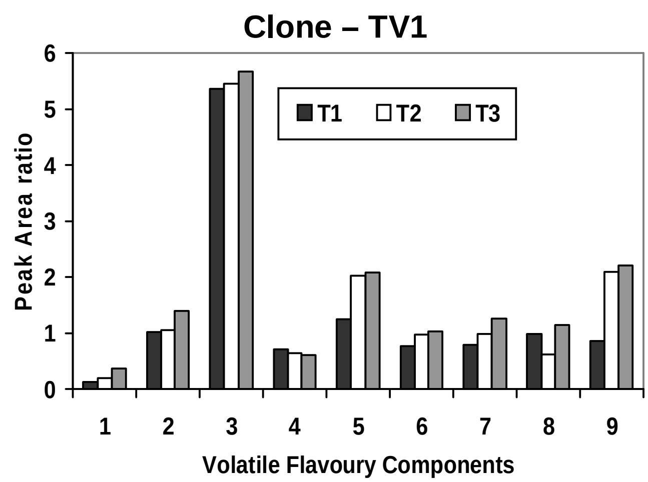

Fig. 2.2. 1-nonanol 2. Phenylacetaldehyde 3.Terpineol 4. Nerol 5.Geraniol 6. α- ionone 7. Benzylalcohol 8. 2-phenylethanol 9. β- ionone.

F* values for** 1. 1-nonanol, 46.75
2. Phenylacetaldehyde, 1.04 NS 3.Terpineol, 0.10NS 4. Nerol, 2.69 NS 5. Geraniol,11.29** 6. α-ionone, 0.80 NS 7. Benzylalcohol, 6.12* 8. 2-phenylethanol, 3.91 NS 9. β- ionone, 11.47**.

Clone – TV26

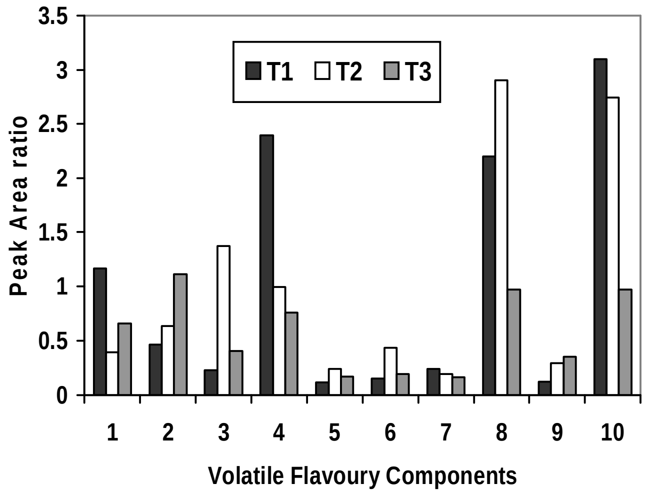

Fig.2.3 1. Hexanal 2. 1-pentanol 3. trans 2-pentenal 4. trans-2-hexenal 5. cis-3-hexen-1-ol 6. Nonylaldehyde 7 Linalool oxide-I 8. Linalool oxide-II 9. Linalool 10. Benzaldehyde.

F* values for** 1. Hexanal , 42.22 2. 1-pentanol, 7.36* 3. trans-2-pentenal, 18.87** 4. trans-2-hexenal, 28.43*** 5. cis-3-hexen-1-ol, 12.17** 6. Nonylaldehyde, 103.47*** 7. Linalool oxide-I, 2.05NS 8. Linalool oxide-II, 53.06*** 9. Linalool, 11.44** 10. Benzaldehyde, 21.10**.

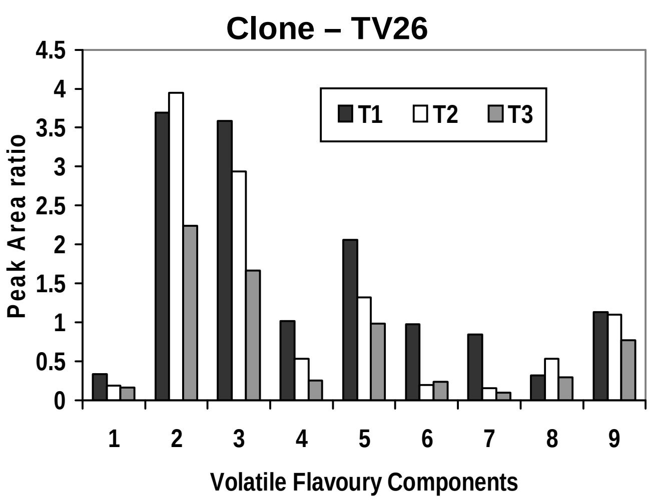

Fig. 2.4 1.1-nonanol 2. Phenylacetaldehyde 3. Terpeneol 4. Nerol 5.Geraniol 6. α–ionone 7. Benzylalcohol 8. 2-phenylethanol 9. β–ionone.

F* values for** 1.1-nonanol, 11.80** 2.Phenylacetaldehyde,27.57 3.Terpineol, 23.14** 4. Nerol, 13.14** 5. Geraniol, 17.39** 6. α-ionone, 17.53** 7. Benzylalcohol, 36.60*** 8. 2-phenylethanol, 22.39** 9. β–ionone, 1.80 N.S

Figs.(2.1-2.4) Effect of different withering conditions on VFC profile of made tea.

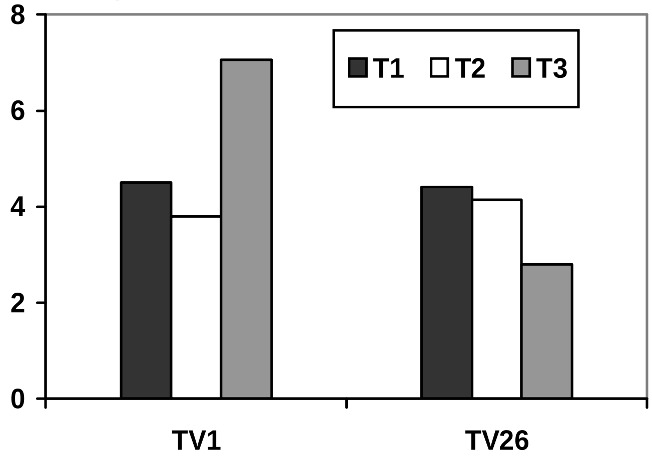

Fig. 3 Effect of different withering conditions on flavour index of made tea.

DISCUSSION:

Catechins are the most important constituents which undergo enzymic oxidation by polyphenol oxidase (PPO) during fermentation stage of black tea processing. EGCG, the major catechin oxidized to the extent of 85-95% during fermentation, has a great impact in the formation of TF and TR (Bhatia 1964). Therefore it is necessary to restrict the catechin degradation during withering which is very much important to improve the black tea quality. In our investigation it appears from the Fig. 1 that catechin degradation is minimum in 100% (T_3) humid condition which is beneficial for the black tea quality. Different degree of physical withers of the initial 5 hrs affects the overall quality of black tea. In the recent past, many attempts had been made by scientists to evaluate the quality of made tea on the basis of TF,TR, brightness etc.(Roberts 1958 and 1962; Robertson, 1992; Ullah and Roy, 1982; Wood and Roberts, 1964; Owuor et al. 1987) The quality, as judges from the TF, TR and brightness of the liquor teas processed under experiment T_3 condition was superior to the other withering conditions which had also a positive correlation with catechin content of withered leaf. Fermentation time is crucial for the formation of quality parameters (Ullah et al.1984) and it is cultivars specific. In our observations (table 2a and 2b) it was appeared that the fermentation time 50 mins. for clone TV1 and 70 mins. for TV26 were optimum. Further this fermentation time for cultivars was similar under all the withering treatments.

Manipulation of withering is very much important for the development of VFC in black tea (Takeo, 1984; Ullah and Roy, 1982). Flavour Index, the ratio of group II to group I VFC (Owuor et. al. 1987; Owuor,1992) is the determining factor for overall aroma of black tea. High amount of group II VFC which are dominated by linalool oxides, linalool, benzaldehyde, phenylacetaldehyde, geraniol, α-ionone etc. is important for high FI and better quality and was found to be the highest in T_3 withering condition for clone TV1, whereas TV26 contained high amount of group I VFC which are dominated by hexanal, t-2-hexenal, cis-3-hexene-1-ol etc. lowering the FI and flavour quality.

Qualitative and quantitative variation of aroma components play a significant role in the evaluation of quality of made tea (Yamanishi et al.1978 Yamanishi,1982). An extensive study were carried out to find out a correlation between flavour Index, quality and withering conditions, more specifically the physical withering under different degree of wet bulb depressions.

Degree of moisture loss achieved during withering has significant influence upon the formation of VFC. Hence regulation of environmental conditions of withering is crucial for overall flavour quality of made tea.

CONCLUSION:

Results of this study indicate that regulation of chemical wither is an important requirement for enhancement of brightness of CTC tea. After plucking, rapid catabolic activities can be reduced by maintaining leaf turgidity for some time. This prevents catechin degradation with consequent effect on brightness.

Different cultivars need to be treated differently during fermentation to optimize product categories. Time of fermentation varies from cultivar to cultivar and hence standardization of processing parameters for different cultivars is a prerequisite to obtain good quality tea. It is important to note that this will help in choosing the right mix of leaf from field with comparable fermenting ability for a batch of processing.

Results also indicated that VFC formation varies significantly between TV1 (Assam hybrid) and TV26 (Cambod hybrid). Geraniol which is an important and characteristic component of black tea was found to be significantly high in TV1 as compared to TV26 manufactured under identical environment.

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