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Preliminary screening of suitable clones for manufacturing green tea from the existing released clones in northeast India

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ABSTRACT

In India, the existing clones and variety of tea plants have been developed for manufacturing of black tea (CTC and Orthodox Tea). But till now no clone and variety of tea has been identified exclusively for manufacturing of Green Tea (steamed/ roasted) so one humble attempt was made to identify tea clones from the existing released clones for preference of manufacturing Green Tea on the basis of their taste and their Polyphenol: Amino Acid Ratio. Selected ten clones representing the China Jat, Assam Jat, Cambod Variety and Industry released Clones of Assam on the basis of their availability and potential quality on CTC and Orthodox Tea were analyzed with their morphological character, chemical analysis and green tea taste by tea taster indicates that TV-1, TV-7, P126 have the best potentiality for manufacturing green tea.

Keywords— *Green tea, Catechin, Epigallocatechin-3-gallate, Antioxidants, CTC (Curling Tearing Crushing), Orthodox, Cambod, Industry released*

1. INTRODUCTION

Demand for green tea is growing in both domestic and international markets. The tea clones and seed stocks grew by the tea industry in N E India are suitable for black tea (CTC/orthodox). The breeding criteria to develop green tea varieties are different from that of black tea varieties. Generally, the small tea leaf variety, also called China *Jat*, is best for making green tea, while the Assam *Jat*, or big leaf variety, is best processed as black teas. The total catechin-Theanine ratio is a good indicator of the taste of green tea. The high ratio of Total catechun to Theanine causes a strong and bitter taste. Whereas tea clones with low Total catechin- Theanine ratio are expected to yield green tea with good taste. Unlike black tea, green tea does not undergo the oxidation (fermentation) process which makes the green leaves to turn brown. Because this step is skipped in the manufacturing of green tea, the chlorophyll remains in the tea leaves which make green tea green. Some of our existing clones that are commonly used for orthodox black tea manufacturing may have the potentiality to yield good green tea.

Green tea production in Assam is at its infancy stages. A few tea processing factories are currently producing them. This is because Assam and may other Indian tea producing states predominantly process black tea (CTC and Orthodox Tea) as their major export market product. As a result, the green teas that exist in the market are thought not to be as good as the ones which are imported into the country. The research was carried out to determine the suitability of ten selected clones from Tea Research Association of India (TRA) in the production of green tea by assaying for biochemical components including Theanine and Total catechins, gallic acid.Two leaves and a bud were obtained from bushes of the selected clones and processed into un-aerated teas before the assays. The results showed that several TRA clones contained high quantities of total polyphenols and total catechins and catechins. Clones which exhibited, low Total catechins were TV-17, TV-1 and TV-7 and clones which exhibited high Theanine were S3A3, P126, TV-17 and TV-7

2. OBJECTIVE OF INVESTIGATION

Evaluation of the chemical constituents (Total Catechin and Theanine content) of the green tea manufactured from the selected clones for their liquor characters.

3. MATERIALS AND METHODS

The China hybrid TV-7, AC hybrid(Assam x China) TV-1, TV-17, TV-31, Assam type TV-21, Cambod type TV-9, TV-23, TV-26, Industry released clones S_3A_3 and P_{126} were evaluated for their potentiality to release as clones for Green tea.

The Green tea manufactured from the selected planting materials was evaluated for the content of Total catechin, caffeine and Theanine using HPLC (Agilent Technology 1260 infinity) following standard protocol.

The transmission color difference of liquor of the Green teas manufactured from a different entry in a different season was measured by using Colorimeter.

Table 1: Collection details of tea samples

Area	Place of collection	Clones/Variety	Month and year of collection
Assam	Borbhetta T.E., Jorhat	TV-1	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	TV-7	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	TV-9	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	TV-17	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	TV-21	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	TV-23	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	TV-26	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	TV-31	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	P126	May, August and October 2018
Assam	Borbhetta T.E., Jorhat	S3A3	May, August and October 2018

3.1 Extraction of total catechins

Extraction of Total catechins was done according to the procedure by ISO14502-2-2005E (2005). Ground tea samples (0.2g) were weighed into graduated extraction tubes, 5ml of 70% hot methanol/water (MeOH) added, stoppered and mixed under vortex (Rotamixer, Huck and Tucker, England). Incubation was done in a water bath at 70oC for 10 min with vortexing after 5 and 10min respectively, cooling to room temperatures and centrifugation (3000rpm) for 10min they followed. A second extraction was done on the residue using 5ml of 70% hot methanol/water, the extracts were combined and made up to 10ml with cold methanol/water (70%).

Determination of Catechins HPLC analysis was done according to the procedure by ISO14502-2-2005E, (2005). One (1) ml of sample was pipetted into a test tube and diluted to 5ml with stabilizing solution (10% v/v acetonitrile with 500µg/ml of EDTA and 500µg/ml ascorbic acid), filtered and loaded into 2ml vials. A Shimadzu LC 20 AT HPLC fitted with an SPD-20 UV-Visible detector and C6, 25cm x 4.6 i.d. column fitted with e Rheodyne precolumn filter (model 7335) was used for determination at 278nm. Gradient elution was employed using the following solvent systems: Mobile phase A (9:2:89 v/v/v Acetonitrile: Acetic acid: EDTA) and mobile phase B (80:2:18 v/v/v

Catechin identification was done by comparing the retention times of samples with those of standards under similar conditions. The standards used included; +C, (-)-EGC, (-) EC, (-)-EGCG and (-)-ECG. Total catechins were expressed as a percentage by mass on a sample dry matter basis and given as a summation of individual catechins as;

$$\% \text{ Total Catechin} = [(\%EGC) + (\%+C) + (\%EC) + (\%EGCG) + (\%ECG)]$$

3.2 Determination of Theanine

Theanine is estimated in HPLC by extracting from the finely powdered sample by brewing with hot water for 5 min using a magnetic stirrer. After filtration of the brew with filter paper, the brew is allowed to cool. The cooled extract is filtered with a 0.45µm membrane filter and 20 µl is injected into a 4.6 mm HPLC reverse phase C18 column with 1.0 ml flow rate using water as eluent and UV detection at 210 nm. Theanine is detected by comparing with Theanine standard through a previously prepared calibration curve (Draft International Standard, ISO/DIS 11287**)**

4. RESULTS AND DISCUSSION

4.1 Analysis of catechin profile

No of samples: 10 different cultivars

Sample type: Green leaf received were deactivated and dried for analysis

Table 2: Analysis of catechin profile

Sample	Caffeine	EGC	+C	EC	EGCG	ECG	Total Catechin
TV1	4.32	2.80	1.17	1.17	7.04	3.63	15.82
TV7	4.74	2.27	0.89	0.83	8.41	3.18	15.58
TV9	6.16	2.84	0.45	0.73	12.46	3.00	19.46
TV17	4.71	2.24	0.82	1.50	7.08	3.76	15.40
S.3A/3	4.16	7.37	0.59	2.55	10.99	4.43	25.92
P 126	4.38	5.79	0.67	1.66	10.57	2.83	21.53
TV23	3.63	4.75	0.74	2.13	10.35	4.50	22.46
TV26	4.04	4.11	0.55	1.03	11.55	2.80	20.03
TV31	3.98	2.44	0.95	0.71	10.80	3.45	18.36

(The values of EGC, +C, EC, EGCG, ECG and Total Catechin are in % dry mass basis)

EGC –Epigallo catechin**, +C –** Catechin, **EC –** Epicatechin, **EGCG** – Epigallo catechin gallate **and ECG –** Epicatechin gallate

Analyzed by Dr.S.Sabhapondit, TRA, Jorhat

Table 2 shows that among the 10 different cultivars analyzed Total Catechin was found to be lowest in TV-17(15.40),followed by TV-7(15.58),TV-1(15.82),TV-31(18.36),TV-9(19.46),TV-26(20.03),P_{126} (21.53),TV-23(22.46),TV-21(22.52),S_3A_3(25.92)

4.2 Analysis of Theanine

No of samples: 10 different cultivars

Sample type: Green leaf received were deactivated and dried for analysis

Table 3: Analysis of Theanine

Sample	Theanine % dry mass basis
TV1	0.56
TV7	1.15
TV9	0.95
TV17	1.15
TV 21	0.82
S3A3	1.83
P 126	1.17
TV23	0.77
TV26	1.04
TV31	0.93

Analyzed by Dr.S.Sabhapondit, TRA, Jorhat Table 3 shows that among the 10 different cultivars analyzed Theanine was found to be highest in S_3A_3 (1.83), followed by P_{126} (1.17), TV 7 (1.15), TV 17(1.15), TV 26(1.04), TV 9 (0.95), TV 31 (0.93), TV 21 (0.82), TV 23 (0.77), TV 1 (0.56)

4.3 Total Catechin to Theanine ratio

Table 4: Total Catechin to Theanine ratio

Tea clones	Total catechin	Theanine	Catechin:theanine
TV 1	15.82	0.56	28.3
TV 7	15.58	1.15	13.5
TV 9	19.46	0.95	20.5
TV 17	15.40	1.15	13.4
TV 21	22.52	0.82	27.5
TV 23	22.46	0.77	29.2
TV 26	20.03	1.04	19.3
TV 31	18.36	0.93	19.7
P 126	21.53	1.17	18.4
S3A3	25.92	1.83	14.2

Table 4 shows that as per ratio of total Catechin to theanine it was lowest in TV 17 (13.4) followed by TV 7 (13.5), S3A3 (14.2), P126 (18.4), TV 26 (19.3), TV 31 (19.7), TV 9(20.5), TV 21(27.5), TV 1 (28.3), TV 23 (29.2)

5. CONCLUSION AND RECOMMENDATIONS

The investigation on 10 different clones of N.E.India has shown that the chemical composition has varied composition some have exhibited higher Total catechin and some have higher Theanine which implies that the clones with lower Total Catechin to Theanine ratio would have lesser bitterness and astringency and thus suitable for Green Tea processing. Individually each assayed tea exhibited a unique combination of biochemical compounds which will uniquely affect the taste of the made teas this is subject for assessment through sensory evaluation. Green tea products generally cost much more than black tea products.

5.1 Recommended clones for green tea

TV-7, TV-17, S3A3. P126

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7. REFERENCES

[1] Quantitative Analysis Of Green Tea Polyphenols In Indian Cultivars Longo, G., 1 Karan, M., 2 Sharma, P. D., 2 Rakesh, D. D.,2 Vyas, S.2 and Vasisht, K.2

[2] Effect Of Microwave And Infrared Radiation On Drying Of Onion Slices Hussain Sorour1 and Hany El-Mesery

[3] Withering timings affect the total free amino acids and mineral contents of tea leaves during black tea manufacturing Saiqa Jabeen, Sahib Alam, Maria Saleem, Waqar Ahmad, Rukhsana Bibi, Farrukh S. Hamid, and Hamid U. Shah.

[4] Amino Acid Assay by Ninhydrin Colorimetric Method Prepared by Nam Sun Wang.

[5] A novel colorimetric determination of free amino acids content in tea Infusions with 2, 4-dinitrofluorobenzene Lin Chen, Qi Chen, Zhengzhu Zhang, Xiaochun Wan.

[6] Analysis of the ditTerentiation of Japanese green tea cultivars using DNA markers. Satom Matsumoto

[7] Analysis of Theanine in Tea Leaves by HPLC with Fluorescence Detection Ye Ying, John W. Ho, Zhen Yu Chen a and Jun Wang
[8] Food and Beverage Application L-Theanine in Green Tea Josh Young MicroSolv Technology Corporation.
[9] Blending of Clonal Tea Leaves with Leaves from Seedlings in Order to Improve the Quality of Made Tea 1V.A Shanmuga Selvan and 2P. Sivasamy
[10] Characteristics of Eight Japanese Tea CultivarsChika Yagi, Namiko Ikeda, and Dwight Sato
[11] Chemical and sensory quality evaluation of newly developed tea clones in Kenya kill Emily Cherono
[12] A diversity of Catechin in Northeast Indian Tea Cultivars Santanu Sabhapondit, Tanmoy Karak, Lakshi Prasad Bhuyan, Bhabesh Chandra Goswami andMridul Hazarika.
[13] Effect of drying technologies on quality of green tea Xiangyang Lin, Lijing Zhang, Hanwu Lei, Hong Zhang, Yanling Cheng, Rongbi Zhu, Roger Ruan
[14] The effect of plant mineral nutrition on yield and quality of green tea (Camellia sinensis L.) under field conditions Dissertation zur Erlangung des Doktorgrades der Agrar- und Ernährungswissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel
[15] The fingerprint of volatile flavour constituents and antioxidant activities of teas from ThailandPatcharee Pripdeevech , Theeraphan Machan
[16] Variations of glycosidic tea aroma precursors, volatlle, 13 -Dglucosidaseactivity and respiration intensity during green tea withering. Xiaochun Wan, Zhengzhu Zhang, Tao Xia
[17] Home-Processing Black and Green Tea (Camellia sinensis) Dwight Sato, Namiko Ikeda, and Tomomi Kinoshita
[18] Identification of Green Tea (Camellia Sinensis (L.)) Quality Level using Computer Vision and Pattern Recognition Han Zhiyi, Chen Quansheng , Cai Jianrong
[19] L-theanine, a natural constituent in tea, and its effect on mental state Anna C Nobre PhD2, Anling Rao PhD2 and Gail N Owen PhD2
[20] Green Tea: Flavor Characteristics Of A Wide Range Of Teas Including Brewing, Processing, And Storage Variations And Consumer Acceptance Of Teas In Three Countries By Jeehyun Lee
[21] Plant genetic resources of beverage crop with Special emphasis on tea and coffee M. Ahmed, M.I. Hossain and M.S.A. Mamun
[22] Anonymous. Tea Digest. Kolkata, India: Tea Board of India, 2000.
[23] Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit. Rev. Food Sci. Nutr. 1997; 37:693-704.
[24] Beecher GR, Warden BA, Merken H. Analysis of tea polyphenols. Proc. Soc. Exp. Biol. Med. 1999; 220:267-270.
[25] Bhatia IS, Ullah MR. Polyphenols of tea IV- Qualitative and quantitative study of the polyphenols of different organs and some cultivated varieties of the tea plant. J. Sci. Food Agric. 1968; 19:535-542.
[26] Bradfield AE, Penny M, Wright WB. The catechins of green tea Part I. J. Chem. Soc. 1947:32.
[27] Bradfield AE, Penny M, Wright WB. The catechins of green tea Part II. J. Chem. Soc. 1948:2249.
[28] Deng ZY, Tao BY. Effect of green tea and black tea on blood glucose, triglycerides and antioxidants in aged rats. J. Agric. Food Chem. 1998; 46:3875-3878.
[29] Ding M, Yang H, and Xiao S. Rapid direct determination of polyphenols in tea by reverse-phase column liquid chromatography. J.Chrom. a 1999; 849:637-640.
[30] Goto T, Yoshida Y, Kiso M, Nagashima H. Simultaneous analuysis of individual catechins and caffeine in green tea. J. Chrom. A 1996; 749:295-299.
[31] Haqqi TM, Anthony DD, Gupta S, et al. Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. Proc. Natl. Acad. Sci. 1999; 96:4524-4529.
[32] Kohri T, Nanjo F, Suzuki M, et al. Synthesis of (-)-[4-3H] Epigallocatechin Gallate and Its Metabolic Fate in Rats after Intravenous Administration. J. Agric. Food Chem. 2001; 49:1042-1048.
[33] Kwanashie HO, Usman H, Nkim SA. Screening of 'Kargasok tea': anorexia and obesity. Biochem. Soc. Trans. 1989; 17:1132-1133.
[34] Larger PJ, Jones AD, Dacombe C. Separation of tea polyphenols using micellar electrokinetic chromatography with diode array detection. J. Chrom. A 1998; 799:309-320.
[35] Lee LB, Ong CN. Comparative analysis of tea catechins and theaflavins by HPLC and capillary electrophoresis. J. Chrom. A 2000; 881:439-447.
[36] Muramatsu K, Fukuyo M, Hara Y. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. J. Nutr. Sci. Vitaminol. 1986; 32:613-622.
[37] Nakabayashi T. Chemical components in tea leaves. In: Nakabayashi T, Ina K, (Eds.) SK, eds. Chemistry and Function of Green Tea, Black Tea and Oolong tea (in Japanese). Kagaku Shuppan. Kawasaki, Japan, 1991: 20.
[38] Oshima Y. Chemical studies on the tannin substances of Formosan tea leaves. Bull. Agr. Chem. Soc. 1936; 12:103.
[39] Poon GK. Analysis of catechins in tea extracts by liquid chromatography-electrospray ionization mass spectrometry. J. Chrom. A 1998; 794:63-74.
[40] Schulz H, Engelhardt UH, Wegent A, Drews H, Lapczynski S. Application of near infrared reflectance spectroscopy to the simultaneous prediction of alkaloids and phenolic substances in green tea leaves. J. Agric. Food Chem. 1999; 47:5064-5067.
[41] Singh HP, Ravindranath SD, Singh C. Analysis of tea shoot catechins: Spectrophotometric quantitation and selective visualisation on two dimemtional paper chromatograms using diazotized sulphanilamide. J. Agric. Food Chem. 1999; 47:1041-1045.
[42] Suchanti R. Tea Market Annual Report and Statistics. Kolkata, India. J. Thomas and company private limited. 2001.
[43] Suganuma M, Sueoka E, Sueoka N, Okabe S, Fujiki H. Mechanism of cancer prevention by tea polyphenols based on inhibition of TNF-alpha expression. Biofactors 2000; 13:67-72.

[44] Vasisht K, Sharma PD, Karan M, et al. Study to Promote the Industrial Exploitation of Green tea Polyphenols in India. ICS-UNIDO, 2003.
[45] Weisburger JH. Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. Food Chem. Toxicol. 1999; 37:943-948.
[46] Yamamoto T, Juneja LR, Chu DC, Kim M. Chemistry and Applications of Green Tea. CRC Press, Boca Raton, New York, USA, 1997.
[47] Zhu M, Xiao PG. Quantitative analysis of active constituents of green tea. Phytother. Res. 1991; 5:239.

