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Biochemical Changes During Withering Process of CTC black Tea Manufacture

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ABSTRACT

Tea is one of the unique non-alcoholic beverages consumed all over the world next to water. During manufacturing process of CTC made tea, withering is one of the important stage where in physical changes taken place besides certain biochemical variations. Current research was carried out the biochemical changes in the green leaves and quality attributes of made tea with respect to extended withering period using "Assam" seedlings and an unique "Chinery" clone, UPASI-9. Results on biochemical constituents of the both withered green leaves and made teas of "Assam" seedlings and UPASI-9 followed identical pattern with certain deviations. Polyphenols transformed rapidly in "Assam" seedlings while it was found to slow in UPASI-9. Processed teas showed irregular pattern in terms of TF, TR, TLC and WE values in both the test materials. TR:TF ratio enhanced in UPASI-9, after 12 h of withering period. Overall results revealed that withering of harvested green leaves till 16 h could be the optimum time to achieve the quality teas with desired flavor. Data generated on withered green leaf constituents and made tea attributes of both the test materials are presented and discussed in detail.

Keywords: Tea, Extended withering, Biochemical parameters, Quality attributes.

INTRODUCTION

Tea (*Camellia* sps.), a non-alcoholic beverage is consumed world-wide from dawn to dusk, next to water, the "Adam's" wine. Classically, tea plants were classified in to two major taxa, i.e., small leaved "China" jat and broad and large leaved "Assam" type. Later, taxonomists positioned the tea plants into three distinct taxa as "Assam", "China" and Cambod" jats (Barua, 1989). Quality of manufactured tea has composite characters depending on polyphenols, catechins, proteins, caffeine, carbohydrates, enzymes and certain intermediary constituents present in the tender

crop shoots[2,3]. Withering is pre-requisite, first step in black tea processing wherein consistency in oxidation during fermentation was ensured[4]. Earlier, Dev Choudhury and Bajaj[5] reviewed the chemistry of withering and later Ullah[6] established the importance of physical withering during black tea manufacture. Both physical and chemical withering gained importance in black tea manufactures in order to achieve the final produce with good aroma and flavor[7,8]. Scanty information was reported on extended withering period with particular reference to specific jat/clone on final produce. In this context, to study the biochemical changes during extended withering period with two distinct varieties and their quality attributes of final produce was envisaged in the present endeavor.

MATERIALS AND METHODS

The present study was carried out at the UPASI Tea Research Foundation, Tea Research Institute, elevated at 1150 m above MSL situated at Valparai 642 127, Tamil Nadu, India. The crop shoots were collected from UPASI Tea Research Experimental Farm; UPASI released clonal tea plant, UPASI-9 and "Assam" seedlings are the test materials. Crop shoots (two to three leaves and terminal bud) collected individually were subjected to the analysis of green leaf constituents and preparation of made tea. During withering process, samples were drawn at 2.0 h intervals till extended withering period (20 h) and subjected to determination of green leaf constituents. All the experiments were conducted between 2015 and 2016.

Determination of green leaf constituents

Crop shoots with uniform physiological maturity were collected from the field grown tea bushes for quantification of green leaf attributes like polyphenols, catechins, amino acids, etc., following the standard protocols. Amount of polyphenols present in the tea leaves (fresh/withered leaves) were quantified in accordance with Dev Choudhury and Goswami[9] while catechin content was determined as per Swain and Hillis[10]. As per the protocol suggested by Moore and Stein[11] the total free amino acids were quantified and the pigments (both chlorophylls and carotenoids) were estimated by colorimetric method reported by Wellburn[12]. Concentration of reducing sugars was determined using the protocol reported by Hedge and Hofreiter[13]. Quantification of lipids was determined gravimetrically in accordance with Ravichandran and Parthiban[14] while caffeine was estimated the method suggested by Ronald and Ronald[15].

CTC black tea manufacture

About 3.0 kg of two to three leaves and a bud were collected individually from field grown "Assam" seedlings and the clone, UPASI-9 and brought to the miniature manufacturing unit. Harvested crop was spread out on wire trays and allowed to wither up to 20 h to achieve a 25-30% decrease in fresh weight. Withered shoots were passed through a CTC (crush, tear and curl) machine for maceration. After maceration, cut "dhool" was spread out in trays for an optimum fermentation period (45-60 min depending on the type of the tea clone). Fermented "dhool" was dried in a mini fluid bed drier at 107°C for 20 minutes. Samples were sorted out, packed individually in polyethylene bags, labelle and stored for analysis of quality constituents[16]. It may be noted that between 2 and 10 h the moisture content was relatively more, manufactured black tea samples showed erratic results and hence the samples drawn from 10 to 20 h at an interval of 2 h were considered in the present study.

Quantification of black tea attributes

Made tea was assessed for its primary quality constituents such as theaflavins (TF), therubigins (TR), high polymerized substances (HPS) and total liquor colour (TLC) using spectrophotometric method[17]. Caffeine content in ublack tea samples was quantified adopting the procedure reported by Ullah *et al.,*[18]. Colour index and briskness index were computed with the values of TF, TR, HPS and caffeine[19], TR, TF ratio was computed with the values of TF and TR of respective samples. Water extract (total soluble solids) of the infusion expressed on dry matter basis was derived in accordance with IS procedure[20] where as moisture content was determined following the IS method[21]. Crude fibre content was quantified following ISO method[22].

Statistical analysis

Data obtained from the triplicate samples and both crop and lean seasons were subjected to statistical analysis, analysis of variance (ANOVA) and the differences that existed among the results were compared with critical difference (C.D.) at five per cent probability. To establish the precision of the

experimental design and its execution, values of coefficient of variation (C.V.) in per cent were also presented individually for each parameter[23].

RESULTS

As withering period increases from 2 to 16 h the polyphenol content of the harvested green leaf declined significantly from 27.78 to 23.42% (Table 1). Extended withering period beyond 16 h exhibited moderate increase in polyphenols. Identical trend was observed in the case of catechins of "Assam" seedling teas. On the other hand, increasing withering period enhanced the amino acid content from 1.24%. Extended withering period showed two fold increase in amino acid content and registered 2.55%. Similar tendency was registered by reducing sugars where it increases from 1.24% at 2 h to 2.24% at 20 hours. Contrarily, both pigments (chlorophylls and carotenoids) reduced significantly from 2.11 to 1.32% as against the extended withering period, right from 2 to 20 hours. Extended withering of harvested crop shoots declined the lipid content significantly at five per cent probability. As for as caffeine is concerned, caffeine content enhances from 2 to 18 h and then declined moderately. Overall results on green leaf constituents against withering period revealed that the optimum fermentation period was found to be 16 hours.

Table 1: Green leaf constituents of "Assam" seedlings

Withering period (h)	Poly-phenols	Cate-chins	Amino acids	Reducing sugars	Chloro-phylls	Carote-noids	Lipids	Caffeine
					(in per cent)			
2	27.78	14.37	1.24	1.29	2.11	0.61	8.27	2.33
4	27.26	14.10	1.35	1.45	1.65	0.60	8.01	2.45
6	26.18	13.95	1.45	1.52	1.60	0.58	7.74	2.55
8	26.14	13.82	1.57	1.61	1.55	0.57	7.51	2.63
10	24.89	13.15	1.61	1.62	1.51	0.55	7.25	2.72
12	23.89	12.61	1.75	1.67	1.44	0.52	6.95	2.78
14	23.73	12.56	1.82	1.75	1.40	0.51	6.50	2.83
16	23.42	12.51	1.94	1.85	1.35	0.50	6.37	3.04
18	24.45	13.08	2.23	1.93	1.35	0.49	6.20	3.18
20	24.88	13.02	2.55	2.24	1.32	0.48	5.87	2.98
S.E.	0.92	0.71	0.46	0.34	0.21	0.04	0.78	0.41
C.D.	0.80	1.93	1.39	0.91	0.67	0.42	0.08	1.43
C.V.	7.74	5.54	3.63	2.67	1.69	0.38	5.72	3.21

As far as made tea attributes of "Assam" seedlings showed corresponding values (Table 2). Thearubigins increased with increasing time of withering period (from 10 to 18 h) significantly at five per cent level and then declined at 20 hours. There was no definite trend in TR and HPS as against extended withering period. However, values of TR:TF ratio dropped from 13.23 to 8.56 as against the extended withering period. The values of TLC increased with increasing withering period. Similar trend was observed in the case of caffeine content as well. Concurrent increase in the CI was noticed against the increasing withering period. Contrary to CI, BI values declined from 33.00 to 21.57 which is significant at five per cent level. Water extract showed definite trend against the extended withering wherein WE increased with increasing withering period and then moderately declined. Crude fibre content declined from 14.19 to 12.79% as against extended withering period.

As withering period increases from 2 to 18 h, the polyphenols declined significantly from 29.32 to 28.18% (Table 3). Identical trend was observed in the case of catechins of "Chinery" clone. Increasing withering period enhanced the amino acid content from 1.26 to 2.76% till 20 hours. Identical tendency was registered by reducing sugars where it increases from 1.40% at 2 h to 2.75% at 20 h as it was observed with "Assam" seedling teas. Both pigments (chlorophylls and carotenoids) reduced significantly as against the extended withering period, right from 2 to 20 hours. Extended withering of harvested crop

shoots declined the lipid content significantly at five per cent probability, from 7.67 to 5.64%. Caffeine is concerned, caffeine content increases from 2 till 18 h and then it declined moderately.

Table 2: Made tea quality attributes of "Assam" seedling teas

Sampling time (h)	TF (%)	TR (%)	HPS (%)	TLC	Caffeine (%)	CI	BI	TR:TF	WE (%)	CFC (%)
10	0.75	9.92	7.54	2.47	2.31	4.28	33.00	13.23	34.19	14.19
12	0.83	10.56	7.78	2.93	2.49	4.52	30.42	12.72	36.96	13.20
14	0.86	9.49	7.77	3.04	2.81	5.29	27.46	9.87	37.21	13.01
16	0.97	9.24	7.46	3.97	3.47	5.81	22.73	9.53	38.43	12.91
18	1.01	9.12	6.49	4.20	3.20	7.36	24.07	9.03	39.19	12.80
20	0.94	8.05	7.42	3.63	3.74	6.51	21.57	8.56	37.89	12.79
S.E.	0.04	0.12	0.08	0.23	0.15	0.56	1.45	-	1.67	0.47
C.D.	0.08	0.23	0.17	0.46	0.31	1.13	2.91	-	3.27	0.92
C.V.	7.29	6.96	4.58	9.80	10.16	8.17	6.45	-	13.01	3.66

Table 3: Green leaf parameters of Chinery tea clone, UPASI-9

Withering period (h)	Poly-phenols	Cate-chins	Amino acids	Reducing sugars	Chloro-phylls	Carote-noids	Lipids	Caffeine
					(in per cent)			
2	29.32	14.52	1.26	1.40	1.55	0.73	7.67	2.53
4	29.31	14.44	1.43	1.42	1.52	0.70	7.28	2.66
6	29.30	14.38	1.53	1.46	1.52	0.65	6.96	2.71
8	29.06	14.32	1.64	1.53	1.48	0.62	6.85	2.75
10	28.92	14.31	1.74	1.55	1.47	0.57	6.65	2.79
12	28.35	14.16	1.84	1.61	1.43	0.54	6.52	2.85
14	28.35	14.12	2.17	1.68	1.38	0.52	6.44	2.90
16	28.31	14.05	2.22	1.96	1.36	0.52	6.43	3.58
18	28.05	13.85	2.59	2.15	1.29	0.51	5.87	2.96
20	28.18	14.06	2.78	2.75	1.25	0.48	5.64	2.93
S.E.	0.15	0.12	0.15	0.09	0.07	0.04	0.27	0.02
C.D. (5%)	0.29	0.23	0.29	0.17	0.13	0.07	0.53	0.04
C.V. (%)	4.60	1.86	4.29	4.05	2.87	8.81	5.54	3.03

Made tea attributes of "China" cultivar, UPASI-9 presented in Table 4. Thearubigins increased with increasing time of withering period (from 10 to 20 h). There was an increasing trend with respect to TR against extended withering period and then declined. Values of TR:TF ratio enhanced from 9.06 as against the extended withering period and the ratio was dropped beyond 16 h of withering. There was no definite trend in HPS values; it was fluctuating. Similar trend was noticed with CI. The values of TLC and caffeine increased with increasing withering period. Concurrent decline in BI was noticed against the increasing withering period. Water extract showed definite trend against the extended withering wherein WE increased with increasing withering period. Crude fibre content declined from 14.03 to 12.81% as against prolonged withering period.

Table 4. Made tea quality attributes of "Chinery" clone, UPASI-9

Sampling time (h)	TF (%)	TR (%)	HPS (%)	TLC	Caffeine (%)	CI	BI	TR:TF	WE (%)	CFC (%)
10	0.87	7.88	6.65	2.85	2.40	5.97	31.79	9.06	34.88	14.03
12	0.83	8.72	6.91	2.92	2.58	5.28	30.45	10.51	36.46	13.29
14	0.86	8.91	7.00	3.05	2.92	5.56	27.45	10.36	37.26	12.99
16	0.91	9.23	7.45	3.41	3.53	5.91	22.74	10.14	38.32	12.93
18	0.94	9.14	6.47	3.43	3.26	7.30	22.71	9.23	39.34	12.76
20	0.94	8.53	7.44	3.64	3.78	5.91	21.55	9.04	40.02	12.81
S.E.	0.02	0.14	0.09	0.13	0.06	0.07	0.58	-	0.82	0.21
C.D.	0.04	0.27	0.17	0.25	0.11	0.13	1.15	-	1.63	0.41
C.V. (%)	4.18	3.52	2.15	2.06	3.74	5.44	8.47	-	10.41	3.19

Considering the polyphenol content of the "Assam" seedlings and UPASI-9 varied between 1.54 and 4.89% as against extended withering period. Degradation of polyphenols was rapid in the case of "Assam" seedlings while UPASI-9 exhibited slow degradation. Alike trend was observed with catechins wherein the variation between "Assam" seedlings and UPASI-9 were very narrow registering the variation ranging from 0.15 to 1.56%. With regard to amino acid content, UPASI-9 was edge over the "Assam" seedling teas. Unlike polyphenols, catechins and amino acids, the values of reducing sugars are fluctuated. Considering the values of total chlorophylls, "Assam" seedlings registered higher values than that of UPASI-9. Contrarily, UPASI-9 possessed marginally higher values of carotenoids than that of "Assam" seedlings. Invariably "Assam" seedlings contained higher amount of lipids than the clonal test material. There was definite pattern in the case of caffeine content with respect to extended withering period.

After drying the fermented "dhool" showed irregular pattern in terms of TF, TR, TLC and WE values both in "Assam" seedlings and UPASI-9. However, TR:TF ratio was increased in UPASI-9 after 12 h of withering period. Till 18 h of withering period, "Assam" seedlings exhibited higher values of HPS than that of UPASI-9. Caffeine content of the made tea samples derived from UPASI-9 had marginally higher values than that of "Assam" seedlings and followed the same pattern of caffeine distribution as in the case of extended withering of green leaf, except at 20 hours. Extended withering beyond 16 h deteriorated the values of colour index while there was no distinct trend in terms of BI between "Assam" seedlings and UPASI-9 as against extended withering time. Marginal deviation was observed in terms of CFC which is not followed regular pattern.

DISCUSSION

Even though in the present study, two different study materials i.e., "Assam" seedlings and "Chinery" clone, UPASI-9 were deliberated, in most of the green leaf constituents and made tea attributes were found to be similar trend except certain deviations. It may be noted that "Assam" seedlings represent plant to plant variation in terms of their phenology, biochemical constituents, physiological performance and quality attributes besides the biomass/economic productivity[24,25,26,27,28]. It has been noted that the "Chinery" clone, UPASI-9 exhibited exclusive performance and there was no plant to plant variation[29].

During the physical withering the plucked tea shoots loss its moisture, the cell sap becomes concentrated and the leaf becomes flaccid. During withering process, the biochemical constituents transformed from one form to another i.e., the breakdown of protein, increase in the amount of amino acids. Increase in the amount of reducing sugars[30,31]. Increase in caffeine content and enhanced cell membrane permeability was also reported earlier[30]. On the other hand, decrease in chlorophyll and carotenoids pigments reported by Wickremasinghe[32]. Harder form of withering resulted in loss moisture exorbitantly in turn reduces the brightness and briskness besides biochemical changes at the stage of withering during black tea manufacturing. Results obtained in the present study revealed that the exhibited withering time enhanced certain constituents like total sugars, amino acids and caffeine to an extent. Both the pigments declined when the withering time was extended while there was no significant difference in polyphenols and catechins which substantiated the earlier results of Sanderson and Perera[34]. It has been established that the increase in the contents of sugars, amino acids and caffeine might be due to higher rate of respiration with evolution of carbon dioxide and water molecules, transformation of biochemical constituents.and consequent loss in dry matter content[33]. Decline in the values of pigments may be attributed to raise in temperature[5]. Quality of tea is a polygenic character and influenced by a number if components either directly or indirectly. Belitz *et al.,*[35] reported that reduction in moisture content during withering can hance the activity of certain hydrolytic enzymes which in turn enhance the values of water extract. Theaflavins is responsible for black tea brightness and briskness. Temperature play an important role in black tea manufacture; during withering process lower temperature can resulted in higher values of brightness and briskness of the tea liquor. Results obtained in the present study established that withering up to 16 h enhances the quality of processed teas and extended withering deteriorated the liquor characteristics and flavor profile.

CONCLUSION

Sixteen hours withering is optimum to achieve good quality processed teas with desirable range of biochemical properties; processing of tea leaves withered within 10 h resulted in poor biochemical properties. Processing or tea leaves beyond 18 h also deteriorated the quality attributes of CTC black teas.

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Conflicts of Interest

The authors declare no conflict of interest.

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