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BIOCHEMICAL CHANGES DURING FERMENTATION PROCESS OF BLACK TEA MANUFACTURE

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ABSTRACT

The quality of black tea is mainly depends on the standard of plucking the green leaf. The most attractiveness of tea as beverage for the main part due to the presence of Polyphenol and caffeine. Young tea shoots are extremely rich in Polyphenol and caffeine which constitute up to 30 percent and 4 percent. Although many of the biochemical transformations occur during the withering phase of tea manufacture, but the most noticeable changes occur during fermentation. Fermentation is an oxidation process by which the Polyphenol in leaf gets oxidized with the help of endogenous enzyme namely Polyphenol oxidase. Before rolling, Polyphenol and Polyphenol oxidase are located in different compartments in the cell wall. When the green leaf gets crushed during rolling, the Polyphenol and the enzyme mixed in the presence of oxygen and the bio-chemical changes takes place. This involves the oxidation of catechins to theaflavins and thearubigins in the presence of polyphenol oxidase, and unsaturated fatty acids and amino acids to volatile flavour compounds, is thought to be the most critical stage in tea manufacture. TF and TR are basically pigments. The end product of fermentation is TF and TR which are responsible for briskness, brightness and color of the tea liquor. Chemically optimum fermentation may be assessed by monitoring the profile of TF content and taking the time required for the production of maximum TF, proper balance between TF and TR is also essential for a good cup of tea. In this study the colorimetric estimation of substances (polyphenols) and the remaining products such as TF, were carried out to determine the OFT. Fermented dhool 60 to 75 minutes showed that the highest tea liquor color, TF, TR, HPS and caffeine were identified.

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INTRODUCTION

Fermentation which is determined by the atmospheric oxidation of tea flavonols by an endogenous polyphenols oxidase is considered as an important stage at black tea manufacture. The oxidized polyphenols are in place of in square brackets, because they are unbalanced compounds which have to return to steadiness by combination of one or more other polyphonic compounds. The standardization of oxidation carried out during fermentation process ensured by this stage reported by (Omiadz *et al*., 2014). Even though many biochemical transformations take place during the withering phase of tea manufacture, but the most noticeable changes happen during fermentation.

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Fermentation, which involves the oxidation of catechins to theaflavins and thearubigins in the presence of polyphenol oxidase, and unsaturated fatty acids and also amino acids to volatile flavour compounds. Quality of brewed tea is based on the process and early experiments of black tea manufacture centered on fermentation. The volatile compounds are formed either by oxidation of flavonal in the presence of polyphenol oxidase (Sanderson—1975) or by direct biosynthesis (Wickremasinhe- 1975). The Catechins are plays a very significant role in the fermentation process in black tea manufacture from green shoots. The biochemical characters are either nature of the raw material used or formed/ distorted during the course of the black tea manufacture reported by (T.Muthumani *et al*, 2013). The bio chemical parameter of green leaves contain the rich quantity of polyphenolic compounds and some other important enzymes. (Jain and Takeo, 1984, Singh and Ravindranath, 1990., and Robertson

1992) The approximate amount of polyphenols in green leaf and black tea are in the range of 28% --35% and 13%-25% respectively (Lunder, 1992). The Polyphenols comprise flavonals, polyphenolic acids and flavonol glycosides. Flavonols or Catechins are the major oxidizing compounds (Ramasamy and Raju 1993). Fermentation, which involves the oxidation of catechins to theaflavins and thearubigins in the presence of polyphenol oxidase, and unsaturated fatty acids and amino acids to volatile flavour compounds, is thought to be the most critical stage in tea manufacture. Quality of brewed tea depended on the process and early experiments in tea manufacture centered on fermentation. The experiment on fermentation process during black tea manufactured and they were reintroduced in the year 1983. These experiments were based on the measurement of theaflavins content with fermentation time and showed that the in-line theaflavins. Use of theaflavins levels in brewed tea to assess the fermentation time gave closer results to sensory evaluation. During the fermentation process, the formation of certain colored compounds, viz., Theaflavin (TF) Thearubigins (TR) and High polymerized substance (HPS) were takes place. It has been reported that quinones formed during enzymic oxidation may oxidise other substances forming volatile compounds, contributing to tea aroma reported by (Sanderson 1973). Floor fermentation facilitates better air contact with the "dhool" and thus beneficial in optimising the fermentation time in relation to ambient fermentation. (Ramamoorthy, 2001). The quality of black tea has composite characters depending on polyphenols, catechins, proteins, caffeine, carbohydrates, enzymes and inorganic constituents (Robertson, 1992). These quality attributes are present abundantly in the tender shoots of the tea plant, that is two or three leaves and a terminal bud that are used for manufacturing of black teas. Biochemical characteristics of green leaves are dependent on rich content of polyphenolic compounds and some other important enzymes (Jain and Takeo, 1984, Singh and Ravindranath, 1990; Robertson 1992). Polyphenols comprise flavonals, polyphenolic acids and flavonols glycosides. Flavonols or catechins are the major oxidizing compounds (Ramasamy and Raju, 1993). In green shoots the extracts EGCG was found to be the major catechins derivatives (Singh *et al*., 1999). The catechins are plays a very important role in the fermentation process in black tea manufacture from harvested crop shoots.

Experimental site: The present study was carried out at the UPASI Tea Research Foundation elevated at 1159MSL situated at Valparai, Tamilnadu, India 642127.The experimental site is located in the Western Ghats, elevated at 1150 MSL.

Sampling: The green tea shoots were collected from clonal tea plants for the analysis of green leaf parameters and also the preparation of made tea sample for quality parameters. In general green tea shoot contains two or three leaves with a terminal bud were selected and subjected for biochemical estimation and FSSAI parameters.

Determination of quality parameters: Black tea samples are manufactured by using miniature unit (UPASI Tea Research Foundation,) for the analysis quality parameters such as TF, TR, HPS and Total Liquor Colour by spectrophotometer method (Thanraj,1988, Thanraj and Seshadri,1990). The FSSAI parameters were analysed by using the standard method (ISO Method and Indian Standard Method).

Black tea extract: Precisely weighed 2.0 gm of made tea sample was taken in 250 ml clean conical flask. To which 100 ml of boiling distilled water was added an air condenser was placed it. The conical flask was kept on boiling water bath maintained at 95 ^{0}C for 10 minutes with alternating swilling. The content of the flask were filtered either glass wool or cotton and then cooled.

Analysis: Bio chemical analysis was based on the method reported by Takeo and Doosawaa (1976). The solvent extraction of made tea extract was done in separating funnels with sufficient shaking at each and every stage. The quality parameters of made tea such as TF,TR, HPS and TLC were calculated from the absorbance values measured from spectrophotometer. Moisture content of the black tea sample was determined by heating the sample (5gm) in an hot air oven at 105^o C following the Indian Standard IS 13853 : 1994. Total soluble solids in black tea were determined extracting the liquor by refluxing method of ISO 9768: 1994. Crude fibre content in made tea sample was determined by removing following ISO method of ISO10226: 1982. Caffeine content in made tea sample was determined extracting the sample with chloroform method reported by (Ullah *et al* 1987).

RESULTS AND DISCUSSION

Fermentation process which is characterized by the atmospheric oxidation of tea polyphenols by an endogenous polyphenol oxidase is considered as an important stage of black tea manufacture. During fermentation process, the formation of certain colored compounds such as Theaflavin (TF), Thearubigin (TR) and High polymerized substances (HPS) are takes place. It has been reported that quinones formed during enzymic oxidation may undergo oxidize, the other substances forming volatile compounds contributing to the black tea aroma characters (Sanderson 1975). Dev Choudhury and Bajaj reported that (1980), the role of different biochemical parameters in green leaf as precursors for a mixture of quality components in tea. Polyphenols and catechins are very significant originator for the production of black tea pigments like theaflavin and thearubigin (Sanderson *et al* 1976). The black tea characters such as brightness, briskness, strength, body and colour of the tea liquor depends upon the oxidation and condensation of polyphenols during the fermentation process reported by (Roberts and Smith,1963, Sanderson 1972, Hilton and Jones 1973).The major products are formed during the oxidation of polyphenols are theaflavins and thearubigins that will decide the total liquor charactestics of black tea, (Roberts 1983). The investigations was established out in connection with the role of polyphenol oxidase on good quality of black tea reported by.(Thanaraj and Seshadri 1990, Dixit *et al* 1981, Obanda *et al* 1992). Dixit 1981 pointed out that the nature and distribution of pigments responsible for quality of black tea formed during the fermentation process are partly governed by the relative actions of polyphenol oxidase and peroxidase. The unoxidized tea flavonols are taken to be responsible for bitterness and astringency in tea liquors. Theaflavins for briskness, brightness and quality and TR and HPS for both color and strength (Sanderson, 1965 and Roberts 1962). Over fermentation results in a liquor of dull color and strength at the cost of quality and aroma. To get the desirable characters in black tea, fermentation to an optimum level is important.

Table 1. Made tea quality characteristics of "China" jat, UPASI-9 in response to variable fermentation time

Fermentation period (min)	Theaflavin (per cent)	Thearubigin (per cent)	High polymerized Substances (per cent)	Total liquor	Caffeine (per cent)
15	0.59	6.04	7.58	1.78	1.83
30	0.62	7.56	8.72	2.40	2.87
45	0.80	9.78	7.37	3.07	3.08
60	0.96	10.67	8.56	3.42	3.11
75	0.93	11.26	9.04	3.69	3.31
90	0.89	11.98	9.22	3.94	2.72
120	0.94	11.24	9.79	3.80	2.53

Table 2. Made tea quality characteristics of "Chinery" clone, UPASI-9: Effect of variable fermentation time on CI, BI and TR:TF

Fermentation period (min)	Colour index	Briskness index	TR/TF	Water Extract (per cent)	Crude fibre content (per cent)
15	3.44	24.38	12.85	45.28	14.94
30	3.56	28.91	12.19	42.26	13.71
45	4.81	25.98	12.23	43.21	15.92
60	4.69	23.59	11.86	38.92	14.94
75	4.07	24.45	12.11	39.81	14.85
90	4.00	28.43	12.10	42.02	15.07
120	3.65	33.57	11.96	45.79	14.98

Table 3. Made tea quality characters of "Assam" seedlings in response to varying fermentation time

Fermentation period (min)	Theaflavin (per cent)	Thearubigin (per cent)	High polymerized Substances (per cent)	Total liquor colour	Caffeine (per cent)
15	0.47	6.05	7.59	1.78	1.89
30	0.56	8.56	8.72	2.40	2.87
45	0.71	9.26	7.36	3.06	3.08
60	0.90	10.69	8.57	3.72	3.55
75	0.83	11.25	9.03	3.41	3.30
90	0.86	11.59	9.23	3.94	2.72
120	0.78	11.42	9.93	3.86	2.23

Table 4. Made tea quality parameters of "Assam" seedlings: impact of varying fermentation time on BI, CI and TR:TF

Fermentation period (min)	Colour index	Briskness index	TR/TF	Water Extract (per cent)	Crude fibre content (per cent)
15	3.44	22.62	16.30	39.64	14.94
30	3.56	24.91	15.02	42.66	13.71
45	4.81	25.90	13.04	44.78	14.70
60	4.67	25.65	13.19	45.22	14.95
75	4.97	24.46	13.55	45.80	13.48
90	4.02	24.05	13.47	42.11	14.18
120	3.83	24.96	12.97	39.64	14.78

To get the best black tea from a particular kind of leaf and maximum desirable characteristics the optimum level of fermentation has to be ascertained. It is therefore considered to find out the optimum fermentation time for clones which differ in their enzyme potential as their substrate levels. The aflavins can be correlated with quality of black tea. (Robert,1962, Hilton and Ellis,1972) have correlated TF content and Total liquor color. Hence it was thought that OFT could be a point at which TF formation is maximum value. Most of the enzymic oxidation reaction carried out due to presence of some main bio-chemical agents involved ie substrate, oxygen and enzyme. In tea fermentation process the substrate ie polyphenols are oxidized by the enzyme, PPO to form quinone. In this process, the products are formed in any enzymic reaction and colored ones such as theaflavins, thearubigins and high polymerized substances were measured by using the suitable wavelengths. TF is responsible for black tea brightness and briskness (Borah *et al.,* 2012). Results showed the TF content was affected significantly by fermentation time. Consequently the effect of fermentation time on tea liquor brightness was non significant. (Borah *et al.,* 2012).

The development of TF and TR is central to the bio chemical reactions during fermentation, but this formation occur only with appropriate combinations of flavanols (Robert, and smith, 1951). These pairs are generally called bio-favonols which differentially reacts with O-Quinones to form TF, and then TR. Theaflavins play the important role of determining the cup quality and brightness of black tea (Roberts, 1962). Total theaflavins in tea liquor constitute about 0.3 to 1.8, of the dry weight of black tea (Robert, and Smith: 1961, 1963). Thearubigin constituting about 30 to 60 percent of the solid in tea liquor affect the color, strength and briskness of brewed tea (Robert and Smith, 1963; Millin *et al* 1969) Thearubigins are also made up of a group of complex compounds after divisible in to fractions (Robert,1962). TR may be mixtures of polymeric proanthocyanidins containing flavanoid residues (Brown *et al*. 1969). The origin and nature of TRs are not very clear and so also the significance of the proportionate increase in TRs with the increase of fermentation period. The color is determined mostly by the ratio of the TF and TR and in a small measure from the presence of flavanotropolones (Roberts:1962) strength is a function of the oxidation of

polyphenols content of the green leaves during fermentation. In general the quality may exist between TF content and the TF/TR ratio, particularly than optical density values, (Takeo 1964) Quality has also been related to briskness (Biswas and Biswas 1971) though quality strength and color may vary according to relative proportions of polyphenols, caffeine thiamine, theogallin and other compounds (Sanderson *et al* 1976). Both TF and TR, the cream is also made up of caffeine (Bhatia, 1964). Caffeine plays an important role in the development of the taste of the beverage, particularly its briskness (Wood and Roberts, 1964). Different types of fermentation are adopted based on the production capacity and market requirement. In general, drum fermentation was adopted in most of the tea factories followed by floor fermentation. Drum cum floor fermentation has found to be better than drum fermentation alone. Floor fermentation facilitates better air contact with the "dhool" and thus beneficial in optimising the fermentation time in relation to ambient fermentation. (Ramamoorthy, 2001). The quality of black tea has composite characters depending on polyphenols, catechins, proteins, caffeine, carbohydrates, enzymes and inorganic constituents reporeted by (Robertson, 1992).Theaflavins can be correlated with quality of black tea (Robert, 1962; Hilton and Ellis, 1972) particularly with TR content and TLC. Crop shoots comprising two leaves and a bud are plucked from the field and brought to processing units where commercial product is manufactured. As cited earlier, processed commercial tea is classified in to three types according to the degree of fermentation viz., unfermented green tea, semi-fermented oolong tea and fully fermented black tea (Xu and Chen, 2002). The basic steps are involved in the black tea manufacture from green shoots are represented as follows: "withering, rolling or cutting, fermentation, drying, sorting and packing (Roberts, 1949). Black tea is manufactured from green leaf and the biochemical constitution changes are associated with the tea processing. (Sanderson, 1975; Barbora, 1992; Ramasamy, 1995; Hudson, 1999).

Conclussion

In this present study the colorimetric assessment of polyphenols and the remaining products such as Theaflavins, Thearubigins, High Polymerised Substances, and Total Liquor Colour and Caffeine were determined. Optimum Fermentation Time was determined. Impact of fermentation time on liquor colour of tea was significant at the 5% level. Fermented cut dhool showed that the highest black tea quality parameters obtained lies between 60 minutes to 75 minutes. The extent of fermentation process can be measured by the level of formation of the black tea quality parameters. The aroma of black tea was found to turn down with fermentation time. Over fermentation time resulted in liquor with dull colour and strength at the cost of quality and aroma. To get the desirable characters in black tea, optimum fermentation time is considered to be important for the good quality of black tea.

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