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Enhancing the quality of naturally oxidized tea with ascorbic acid

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Abstract— *Tea globally the most popular refreshing beverage, processed from the leaves of Camellia sinensis is evaluated organoleptically. While the biochemical potentials are fixed in the leaves, it is made palatable through processing. But for the rich polyphenols and caffeine in tea, it would not have been a beverage. Catechins are about two thirds of polyphenols undergo oxidation in the presence of the polyphenol oxidase to produce polyphenolic pigments namely theaflavins (TF), thearubigins (TR) and other polymerization compounds. The level of these compounds and their balance in the tea liquor determine the quality of black tea. Among these theaflavin plays a pivotal role and is directly proportional to the quality of liquor. Hence, an attempt has been made to increase the theaflavin levels and other quality attributes of the liquor through addition of ascorbic acid. Native level of ascorbic acid is very less in tea leaves and negligible in processed tea. Ascorbic acid when added during oxidation process reacts with catechins to produce stable secondary polyphenols. Ascorbic acid addition increases the formation of theaflavins from 1.23 to 1.77%. Moreover further polymerization of polyphenols to other undesirable complexes is arrested due to reduced oxygen uptake. Ascorbic acid addition at optimum level does not change the inherent quality and related attributes of the tea liquor. The stability and total liquor colour of the liquor is increased from 3.82 to 4.72%. As increasing the native level of ascorbic acid is difficult, external addition of ascorbic acid in processing is to be explored. The pH and EC value does not change up to addition of 2500 ppm concentration of ascorbic acid. Addition of ascorbic acid is enhancing the overall quality of black tea at concentration of 2500 ppm. Ascorbic acid is also being a tool in biosynthesis of several secondary polyphenols such as theaflavins thearubigin.*

Keywords— ***Black tea, polyphenols, catechins, oxidation, theaflavins, ascorbic acid.***

I. INTRODUCTION

Tea (*Camellia sinensis*) is the most popular drink in the world next to water. The major types of tea are green and black tea and other than this there are some specialty teas like white tea, yellow tea, etc. Polyphenol content in black tea is liquor colour and brightness could be considered as reliable quality parameters (Arachchi *et al* 2011) . Tea leaves contains polyphenols around 30% on dry weight and polyphenols have catechins as their major constituent. Oxidation of catechins is a very important process during black tea production (Matsuo *et al.,* 2008). These catechins are converted to theaflavin, thearubigin and high polymerized substances during oxidation with the help of the polyphenol oxidase. Here the catechins are oxidized and the tea is called black tea (fully oxidized tea) (Hilal and Engelhardt., 2007; Ravichandran and Parthiban 1998). The polyphenol oxidase in the leaf is deactivated, there is no oxidation of catechins during the process and the tea is called Green tea (unoxidised tea) (Hilal and Engelhardt 2007). Green tea process consists of mainly three steps steaming/panning, maceration of leaf and drying. The plucked leaves are immediately steamed or panned for deactivating the enzyme polyphenol oxidase (PPO) and then the leaves are rolled (maceration) and dried to moisture content below 3%. In this process the polyphenols and catechins are retained. Black tea process is mainly a four step process involving withering, maceration, oxidation and drying. The plucked shoots are allowed to wither for a duration varying from 12 to

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20 hrs in order to remove around 5 to 20% moisture and concentrate the cell sap there by enabling it to be macerated without any loss of juices. During withering flavour compounds are developed due to chemical changes. After withering leaves are ruptured and macerated. After maceration the cut dhool is oxidized, which is indicated by the colour

change from green to coppery brown. Finally the oxidized tea is dried to arrest the oxidation process and reduce the moisture below 3%. Based on the method of manufacture, generally there are two types of black tea processing namely Orthodox process (traditional method) for more flavour and CTC process (crush, tear, curl) for more colour and strength in the liquor. Tea is evaluated organoleptically. This is correlated with bio chemical constituents present in tea. Green tea is rich in polyphenols since they are not oxidised during the process and catechins comprise two thirds of polyphenols. These unoxidised catechins are the quality indicators of green tea. In black teas during oxidation catechins are converted to theaflavins, thearubigins and high polymerized substance due to the activity of enzymes (Kim. *et al*., 2001; Ansari *et al*., 2011). Theaflavin and Thearubigins are the quality indicators for black tea as these two compounds are responsible for the taste, colour, briskness brightness and strength of the black tea (Owuor and Obanda., 1998). Given below are major catechins present in tea and their conversion (Ngure *et al.*, 2009).

These catechins are oxidized in the presence of enzymes to form the pigments theaflavin and thearubigin. This oxidation reaction is a continuous process. Initially catechins are dimerized to form theaflavin and further oxidized to trimer form of thearubigin and polymerized to form high polymerized substances (Roberts 1941). Theaflavin plays a major role in fixing the quality of tea. Generally tea retaining higher amount of theaflavins are considered as better quality tea. A lot of research has been conducted for retaining or increasing the theaflavin levels in tea. Here edible acids (specifically ascorbic acid) are used to obtain higher level of theaflavin. The native level of ascorbic acid in tea leaves is around 0.2% and act as an enzyme. After processing black tea is contains negligible amount of ascorbic acid (Hussian *et al* 2006; Senthilkumar and Ramesh Kumar 2004).

The effects of studies on influence of ascorbic acid on the oxidation of tea are being continued (Deijs 1940). Ascorbic acid is useful for producing higher amount of theaflavin, increasing thearubigin and decreasing the polymerization. The amount of theaflavin and thearubigins are directly related to quality of tea where as higher amount of polymerized substances have an adverse impact on the quality of tea. The experiment is about external addition of ascorbic acid during processing and its impact on quality of tea. Tea was analysed for theaflavins, thearubigins, high polymerised substances, total liquor colour, total polyphenols, total catechins, pH, electrical conductivity and FSSR parameters before and after the external addition of ascorbic acid.

II. MATERIALS AND METHODS

A. Materials

IBMK (iso butyl methyl ketone), n-butanol, disodium hydrogen phosphate, sodium carbonate, folin & Ciocalteu`s Phenol reagent, Sulfuric acid, hydrochloric acid, sodium hydroxide, alcohol, acetone, catechin, gallic acid, caffeine and ascorbic acid.

B. Tea process

Leaf of UPASI – 9 tea clone were plucked and allowed to wither for 16 hours. The withered leaf was macerated with CTC roller and allowed to oxidize for 90 min. After maceration, the tea leaf was divided to eight parts. One part was allowed to oxidize normally and the other seven parts was sprayed with ascorbic acid at 500, 1000, 1500, 2000, 2500, 5000 and 10000 ppm levels. The dhool (macerated tea leaf) after oxidation was dried for removal of moisture and deactivation of enzymes. The tea was processed at UPASI

mini factory, Glysdale farm, Coonoor, The Nilgiris, Tamilnadu, India. The processed made tea was graded and BOP (Broken Orange Pekoe) grade was selected for laboratory analysis.

C. Analysis

Analysis was carried in UPASI Tea Research Foundation, Regional centre, Coonoor, The Nilgiris, Tamil Nadu, India, a NABL (National Accreditation Board for Testing and Calibration Laboratories) accredited laboratory for chemical testing as per ISO/IEC 17025:2005. All analytical results were calculated on dry matter basis (DMC).

D. Quality Parameter Analysis

Liquor

2 g of BOP grade tea was added to 100 ml freshly boiling water and steeped in boiling water bath for 10 min at above 85^0C. Brewed tea was filtered through cotton and liquor taken for analysis. 25 ml of tea brew was shaken with

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25 ml of IBMK in separating funnel and allowed to separate, to organic (A) and aqueous layer. 10 ml of aqueous layer was shaken with 10 ml of Butanol and allowed to form organic (B) and aqueous (D) layer. 10 ml of organic layer (A) solution was shaken with 10 ml of 2.5% disodium hydrogen phosphate solution and allowed to separate to organic (C) and aqueous layer. 1 ml of each A, B, C, and D layer was pipetted out to 9 ml 45% ethanol in boiling tube. 1 ml of tea brew was pipetted out to 9 ml of distilled water containing boiling tube (E). The optical density (OD) of all the solutions was determined in UV spectrophotometer (GBC 918. Australia). The OD of solution E found at 460 nm represents total liquor colour of tea (TLC). OD at 380 nm of A, B, C, D solutions were determined. Here A, B and C represent Thearubigins (TR), C represents Theaflavins (TF) and D represents High Polymerised Substance (HPS). Concentration of TF, TR, HPS and TLC was calculated from the absorbance values as given below. The calculation factors include molar extension coefficient and dilution (Roberts and Smith., 1963; Tea board., 1995). The results are represented on dry basis.

$$TF\% = \frac{C*4.313*100}{DMC} \qquad TR\% = \frac{(A+B) - C*13.643*10}{DMC}$$

$$HPS\% = \frac{D*13.643*100}{DMC} \qquad TLC = \frac{E*10*100}{DMC}$$

Dry matter content (DMC)

The procedure for determining dry matter content is given below. Weight the empty bottle (A) g, Bottle with 5 g tea sample (B) g and Bottle with 5 g tea sample after 16 hours drying (C) g.

$$DMC\% = \frac{(C-A)*100}{(B-A)}$$

Total Catechins

0.2 g ground tea sample was extracted with 5 ml of 70% hot ethanol in water bath at 70^0 C for 10 min, then centrifuged at 3500 rpm for 10 min and filtrate decanted in 10 ml. This process was repeated twice and made up to mark. 2 ml of filtrate was pipette out into 100 ml. SMF and made up to mark. 2 ml of aliquot was pipette out in a boiling test tube and 6.5 ml of 1% vanaline solution (70% Sulphuric acid) was added. 1.5 ml of water to this solution and 30 min were allowed for development of colour. After colour developments optical density was found at 500 nm in spectrophotometer (GBC 918, Australia). The same process was done for standard catechins 10, 20, 30, 40, 50 ppm standard solutions.

$$\text{Total Catechins \%} = \frac{\text{Graph reading}*12.5}{\text{Weight}* \text{DMC}}$$

Total Polyphenols

0.2 g of ground tea sample was extracted with 10 ml of 70% hot methanol in water bath at 70^0C for 10 min, and then centrifuged at 3500 rpm for 10 min and the filtrate decanted to 10 ml SMF. This process was repeated twice and made up to mark. 1 ml of filtrate diluted to 100 ml in SMF. 1 ml of this solution was pipette out to boiling tube, 5 ml of Folin-Ciocalteu: water reagent (1:10) added, after 5 min. 4 ml of 7.5% sodium carbonate solution was added and allowed to remain for 1h for colour development. OD was found at 765 nm in spectrophotometer (GBC 918, Australia). The same process was done for standard Gallic acid 10, 20, 30, 40 and 50 ppm solutions.

$$\text{Total polyphenols \%} = \frac{\text{Graph reading}*10}{\text{Weight}* \text{DMC}}$$

Caffeine

1 g ground tea sample was soaked in 5 ml liquor ammonia in a separating funnel for 5 min. 25 ml of chloroform was added to separating funnel, shaken well and allowed to settle for 30 min. After settling chloroform solution was transferred to 1% potassium hydroxide in separating funnel, extracted and allowed to separation for 30 min. The extracted caffeine in chloroform solution was filtered through anhydrous sodium sulphate to 100 ml. This process was repeated thrice to made mark; 2.5 ml of chloroform solution was diluted to 50 ml. The standard caffeine at 1, 2, 4, 6, 8, 10, 20 ppm was prepared in chloroform. OD was found at 274 nm in spectrophotometer (GBC 918, Australia).

$$\text{Caffeine \%} = \frac{\text{Graph reading}*20}{\text{Weight}*\text{DMC}}$$

Total Lipids

1 g ground tea sample was soaked in 1% NaCl solution in a separating funnel and extracted with chloroform

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and allowed to separate for 30 min. The chloroform solution was filtered through anhydrous sodium sulphate to pre weighed china dish (L_1). This process was repeated thrice. The china dish containing chloroform was evaporated in water bath and dried in hot air oven for 30 min, cooled and weighed (L_2).

$$\text{Total Lipids \%} = \frac{(L_2-L_1)*100*100}{\text{Weight}*\text{DMC}}$$

Taste

The 2% BOP grade tea brew was prepared with five minutes steeping time. The brewing tea details was hide and evaluated by a professional taste expert from taster`s office, Coonoor, The Nilgiris, Tamil Nadu, India. The score was given out of ten.

III. RESULTS AND DISCUSSION

A. Theaflavins

The role of theaflavins in improving quality lies in their effect on the brightness and briskness of tea brews, as well as the coppery colour of the infused tea (Ramaswamy 1986; Peterson *et a*l 2005). All these characteristics increase the value of tea. Theaflavin content contributed positively toward valuations for tea quality (Taylor and Francis 1995; Ansari *et al* 2011; Liang and Yu 2001).The experimentshows a clear picture on the importance of ascorbic acid in increasing the level of theaflavin in tea. The results were indicating that tea leaf containing high amount of ascorbic acid had better quality. Increasing the ascorbic acid level externally will have a positive impact on tea quality (Table1). The ascorbic acid added during oxidation act as an enzyme dehydroascorbidase (Tanaka *et al* 2010). Ascorbic acid slows down the formation of thearubigins and high polymerized substances and delays further oxidation of theaflavins. Ascorbic acid reacts with epigallo catechin gallate to form ascorbyl epigallo catechin gallate, which accelerates the theaflavins (monomer) formation and slows down further oxidation. Gradually increasing the ascorbic acid concentration up to 10000 ppm increased the theaflavin levels without any adverse effect in the tea.

B. Thearubigins

The formation of thearubigin is linked to quality characteristics of black tea (Obanda *et al*., 2004). Color and strength are related to thearubigin content, one of the components of thearubigins is theaflavins and it is known that during oxidation the theaflavins reach a peak after which they are believed to undergo further oxidation to produce thearubigins (Taylor & Francis., 1995; Ansari *et al*., 2011). The analytical result shows that thearubigins level increase with increase in concentration of ascorbic acid upto 2500 ppm. Higher concentrations gradually decrease thearubigins (Trimer). Initially the thearubigins levels are increased due to the slowing down of the reactions when ascorbic acid is added. When the addition of ascorbic acid exceeds 2500 ppm, ascorbic acid reacts directly with catechins to produce more theaflavins and hence thearubigins is reduced (Table1). Increase of thearubigins is also impact tea quality positively. The results of the laboratory analysis indicate that the thearubigins levels increase with addition of ascorbic acid, implying a positive influence in quality of tea liquor.

C. High Polymerized Substance

High polymerised substances (HPS) are the products formed due to polymerization during the oxidation process. Increased HPS formation during oxidation had a similarity to the pattern of the colour production (Hafezi *et al* 2006). Generally high polymerized substances have a negative influence on tea quality. Hence higher levels of polymerized substance make the liquor cloudy. Higher amount of HPS indicates that the oxidation process is not proper. The results of the study indicate decrease in the HPS level with increasing ascorbic acid concentration (Table1). Ascorbic acid addition reduces the formation of HPS by slowing down the oxidation process. Addition of low concentration of ascorbic acid reduces polymerisation. Addition of concentrations above 2500 ppm ascorbic acid marginally reduces HPS due to direct reaction of ascorbic acid with catechins. The results indicate a decrease in HPS due to addition of ascorbic acid which is good for the quality of tea.

D. Total Liquor Colour

Colour is an important quality attribute (Obanda *et al* 2004; Bokuchava *et al* 1980). There is an increasing demand for natural food colour all over the world. Tea is a source of natural colour (Baruah *et al*., 2012). The characteristic colour of black tea is formed during its oxidation process. During this process, the colourless catechins which are abundant in fresh leaves are oxidized both enzymatically and chemically to give two major groups of pigments, theaflavins and thearubigins.The colour of liquor increases with the addition of ascorbic acid upto a concentration of 2500 ppm as

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concentrations up to this level increases theaflavins and thearubigins (Table1).

E. pH and EC

Lower pH in tea is associated with an apparent increase in theaflavins (Subramanian *et al* 1999 & Vuong. *et al* 2013). The presence of ascorbic acid may partially prevent the degradation or epimerization (Chen *et al* 1998). The ascorbic acid inhibit oxidation reaction (Zimmermann & Gleichenhagen 2011).The experiment shows low concentration of ascorbic acid did not have any change in pH & EC. At higher concentration exceeding 2500 ppm, pH was reduced by 0.19 units at 5000 ppm and by 0.39 units at 10000 ppm (Table1). Higher concentration of ascorbic acid increased the electrical conductivity. Theaflavin is more stable in medium than alkaline range of pH (Su 2011).

TABLES

Table 1. Liquor Parameters (Brew)

Con. Ascorbic acid (ppm)	**Theaflavin (TF) %**	**Thearubigin (TR) %**	**High polymerized substance (HPS) %**	**Total liquor colour**	**pH**	**EC (ds/m)**
Control (0)	1.23	10.30	9.71	3.85	4.88	0.95
500	1.28	11.23	8.84	4.83	4.88	0.95
1000	1.33	11.42	8.75	4.33	4.91	0.93
1500	1.42	11.68	9.67	4.53	4.89	0.94
2000	1.48	11.89	9.54	4.42	4.91	0.95
2500	1.52	12.03	9.41	4.72	4.89	0.95
5000	1.58	11.47	8.11	4.44	4.69	1.22
10000	1.74	10.54	7.08	4.44	4.28	1.40
CD at P= 0.05	*0.02*	*0.05*	*0.06*	*0.03*	*0.01*	*0.02*
CD at P= 0.01	*0.03*	*0.06*	*0.09*	*0.04*	*0.01*	*0.03*

Table 2. Biochemical parameters (Processed tea)

Con. Ascorbic acid (ppm)	**Total Polyphenols%**	**Total Catechins %**	**Total Lipids %**	**Caffeine %**
Control	16.77	6.52	8.01	2.42
500	16.70	6.41	7.85	2.43
1000	16.50	6.39	7.76	2.42
1500	16.38	6.35	7.27	2.44
2000	16.23	6.29	7.18	2.45
2500	16.05	6.07	6.21	2.44
5000	14.52	4.62	6.28	2.43
10000	13.38	3.55	5.94	2.42
CD at P= 0.05	*0.07*	*0.05*	*0.07*	*0.09*
CD at P= 0.01	*0.09*	*0.07*	*0.09*	*0.13*

F. Total polyphenols and Catechins

During the course of oxidation, the polyphenols are rapidly converted to pigments (Harbowy and Balentiene., 1997; Sang *et al*., 2011). The lower the pH and temperature the more stable the tea catechins are during processing and storage. Tea catechins are stable in acidic system (Ananingsih *et al*., 2013). During fresh tea leaves are crushed at the initial stage, the four major catechins are enzymatically oxidized and the resulting quinones undergo complex chemical changes. The composition of the oxidation products of tea catechins is extremely complex (Tanaka and kouno 2003). There is a slight decrease in polyphenols and catechins with addition of low concentration of ascorbic acid (upto 2500 ppm). At addition of higher concentration ascorbic acid (5000 and 1000 ppm) catechins react with ascorbic acid to form theaflavins (monomer), reduce formation of thearubigins (dimer) and hence there is a notable decrease in the level of polyphenols and catechins (Table 2).

G. Lipid and Caffeine

Generally a lipid is negatively correlated to tea quality (Ganesan and Ramasamy., 1996). In this study addition of ascorbic acid decreased the lipid levels. High lipid

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leads to Pacha taint in tea (Ganesan and Ramasamy., 1996). Ascorbic acid gradually decreased the lipids level and caffeine is responsible for the briskness (Borse and Rao 2012). Caffeine is an alkaloid group compound which is naturally present in tea leaves. It does not involve in the oxidation process (Kim *et al*., 2001). No change in caffeine level was observed during this study. Caffeine content was almost the same with or without addition of ascorbic acid (Table1).

Table 3. Taster Evaluation

Con. Ascorbic acid (ppm)	Infusion	Colour	Strength	Briskness	Other comments
500	7	5	7	5	-
1000	7	6	7	6	Good infusion
1500	7.5	6	7	6	Good liquor
2000	8	7	8	7	Good liquor
2500	8	7	8	8	Good liquor
5000	9	5	6	5	Slight sour
10000	9	5	5	5	sour

H. Taste

Tea contains a number of biochemical components, some are responsible for colour, and some are responsible for taste of the liquor (Baruah *et al*., 2012; Liang *et al*., 2003). These biochemical parameters directly correlated to taste of the tea (Ngure *et al*., 2009). Higher amount of polymerized substance present in tea give undesirable taste. Hence during oxidation process polymerization should be as minimum as possible. Theaflavins are responsible for astringency and briskness. Thearubigins gives strength and good mouth feel. Addition of ascorbic acid gradually increased the taste related compounds (Table3).

IV. CONCLUSION

This study clearly shows that addition of ascorbic acid during oxidation process increases the overall quality parameters in tea. Tea is stable in acidic conditions and addition of ascorbic acid reduces catechins degradation in brew and processed tea. This study indicates the role of ascorbic acid in increasing the quality of tea. Hence, increasing the native level of ascorbic acid in tea leaf will help to increase the tea quality parameters naturally. Addition of ascorbic acid is enhancing the overall quality of black tea at concentration of 2500 ppm. This study gives gate way for further research on extraction of theaflavin, isolation of flavour compounds and increasing shelf life of tea brew.

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