A Unique Method Of White Tea Preparation With Clonal Teas And Evaluation Of Its Phenolic Bio-constituents

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ABSTRACT

An attempt has been made to evaluate introduction of microwave steaming in white tea processing against conventional processing technique. Microwave steaming saved ample time in production of white tea. Comparative analysis of biochemical constituents of final produce revealed that the total polyphenol and catechins were significantly higher in modified method of white tea processing than that of conventional technique. Tested tea clones exhibited variations in terms of polyphenols, catechins and its derivatives. Data revealed that no tea clone follow identical pattern with regard to compositional distribution of catechin fractions. Results obtained in the present endeavour substantiate that the tea clones, CR-6017 and TTL-2 represents high grown quality tea clones that can be utilised for white tea processing followed by UPASI clones.

Key words: white tea processing, polyphenols, catechin fractions, catechin index

INTRODUCTION

Tea, one of the most popular beverages, manufactured using tender crop shoots harvested from cultivated species of *Camellia* (viz., *Camellia sinensis* (L) O Kuntze, *Camellia assamica* (Masters) Wight and *Camellia assamica* ssp. *lasiocalyx* (Planch ex Watt) Wight). According to the manufacturing methods, the final produce categorized as unfermented green tea, partly fermented oolong tea and completely fermented black tea besides specialty teas otherwise known as white tea or silver tips (Sharangi, 2009; Unachukwu *et al*., 2010). Consequently, these teas are varied in their biochemical constituents, quality attributes and sensorial properties (Del Rio *et al*., 2004; Hilal and Engelhardt, 2007; Mizukami *et. al*., 2007; Wang and Ho, 2009).

In recent years, white tea has been gaining more attention among the tea consumers in worldwide due to its health benefits. Unlike the green tea/black tea, white tea is not rolled or fermented, resulting in a 'lighter' flavor characteristics and its tea brew is pale yellow in colour. In general, unopened large buds with dense pubescence and/or unexpanded first leaves of flush is used for white tea preparation (Hilal and Engelhardt, 2007). Pubescence or epidermal hairs on the surface of the unopened buds imparts shining white to silver colour to minimally processed white tea/silver tips which retained entire amount of phytochemicals, intact (Jenny and Mao, 2012; Shitandi *et al*., 2012). Polyphenols, particularly catechins and its derivatives are known for their wide spectrum of biological activities like antioxidant, antiviral, anticancer, antibacterial, antifungal, antitoxoplasmal, antitrypanosomal, anticoccidial, antinematodal and antihelminthic (Cooper 2011; Fassina *et al*., 2002; McKay and Blumberg, 2002; Yang *et al*., 2009)

Extensive studies were conducted on manufacturing methods (Ramaswamy *et al*., 2000; Anon., 2007) and quality characteristics of south Indian black teas (Venkateswaran *et al*., 2002; Muthumani and Senthilkumar, 2004; Senthilkumar *et al*., 2011). Only scanty reports are available on white tea processing that too involved prolonged withering (required 4-5 days to obtain final product with 3-4% moisture) (Ramamoorthy, 2006). There are no attempts made to reduce the white tea processing time and its biochemical composition particularly polyphenols and catechins. In this context, an attempt has been made to reduce white tea processing time by introducing microwave drying/steaming and in white tea processing and compared with conventional processing in terms of polyphenols and catechins besides catechin fractions using certain popular south Indian tea clones.

MATERIALS AND METHODS

Plant material:

Five tea clones, UPASI-9, UPASI-20, TTL-2, CR-6017 and TRI 2043 were selected for the study. Among them UPASI clones, (UPASI-9 and UPASI-20) were released by UPASI Tea Research Foundation (formerly UPASI Tea Research Institute), Valparai, Coimbatore District, Tamil Nadu, India. UPASI-9 plants are known for its productivity and drought tolerant features planted widely in south Indian tea plantations while UPASI-20 is highly pubescent and quality related tea clone and both the clones expresses more affinity towards "Chinary" characteristics in terms of morphology. High quality tea clone, TTL-2 is identified and released by R&D Department, Tata Tea Limited, Munnar, Kerala, India which belongs to "Cambod" variety. The estate election, CR-6017 ("Cambod" jat) known for its inherent quality attributes selected from one of the tea estates in The Nilgiris (Craigmore Tea Estate), Tamil Nadu, India. TRI-2043, highly pubescent and average quality clone released by Tea Research Institute of Sri Lanka. These plants were grown in R & D Experimental Farm of Kanan Devan Hills Plantations Company Limited, Munnar, Idukki District, Kerala. The experimental farm located at an altitude of 1500 m above mean sea level. All tea husbandry practices including fertilizer application and plant protection measures were adopted in accordance with UPASI recommendations (Durairaj *et al*., 2015) and estate practices.

Sample collection:

Unopened buds collected afresh from the above said tea clones individually during winter season. Handpicked buds were collected/placed in a basket in order to avoid damage to the harvested buds and brought to the laboratory for white tea preparation. It may be noted that damaged to the harvested materials will hasten the polyphenol oxidation.

White tea preparation*:*

Conventionally, silvertips/white teas are prepared using growing unopened buds. White tea does not require panning, rolling, etc. They were dried under natural sunlight which allows the tips retain their covering of velvety silver colour. In the sun drying process the buds could be spread on nylon nets and allowed to dry at an optimum temperature till attain silvery colour. At times the core moisture will be removed by using electrical heaters during night times (Anon., 2007).

In the present study, handpicked tea buds were spread on a mini withering trough equipped with a fan at the bottom and with nylon net on the top. Ambient air was blown for 12 to 16 hours in order to reduce the moisture content. Withered buds were processed in a microwave oven/ steamed for 10 minutes to inactivate the enzyme reactions/oxidation. Further, these were oven dried at 60^{o}C for 3 – 4 hours to reduce the moisture to 3-4%. It is noteworthy that in this modified method of white tea preparation undue delay may be avoided, White teas processed both the methods were stored properly and subjected to biochemical analysis.

Biochemical quantification:

The method described by the International Organization for standardization (ISO: 14502-1, 2005) was followed for sample extraction; quantification of total polyphenols was carried out by adopting Folin-Ciocalteu phenol reagent method where gallic acid used as standard and the polyphenol content of the samples expressed as gallic acid equivalents. Total catechins was estimated by the method described by Swain and Hillis (1959) using (+) catechins as standard. Catechin fraction quantification was done following ISO 14502-2 (2005) method. Technical standards (epigallo catechin, (+) catechin, epicatechin, epigallo catechin gallate and epicatechin gallate) procured from Sigma Aldrich, USA were used for preparation of calibration curve. Individual catechin fractions were computed and presented as per cent corresponding to catechin fraction/g dry weight.

Data analysis:

Statistical comparisons of the mean values of five replicates were performed by general linear model. Generated data were statistically analyzed (factorial design) and the differences that existed among the results were compared with critical difference (C.D.) at five per cent probability. For the compositional data analysis of catechins computation of different catechin fractions were used according to (Saravanan *et al*., 2005; Himangshu Deka *et al*., 2021). Gallated catechins are sum of epigallocatechin gallate and Epicatechin gallate while non gallated catechins derived from epicatechins and epigallocatechins. Dihydroxylated catechins (DHC) are derived by summing up of epicatechin and epicatechin gallate while trihydroxylated catechins (THC) obtained by summing up of epigallocatechins and epigallocatechin gallate. Catechin index = DHC/THC.

RESULTS AND DISCUSSION

Introduction of intermediate microwave/steaming process in the conventional method reduced time in obtaining the final product. In the modified process of white tea manufacture, it requires 16-20 hours whereas the conventional processing took more time to achieve 3-4% moisture level of the final produce. Visually, appearance of the white tea obtained in the both the methods are identical; however, varietal difference was noticed among the final produce obtained from different tea clones. This is attributed to the degree of presence of velvety leaf hairs. It is noteworthy that the clones selected were possessed moderately dense to highly dense pubescence on lower surface the unexpanded first leaf and the growing buds. Irrespective of processing methods, the clones exhibited wide variation in total free polyphenol content (Table 1). Among the clones, TTL-2 and CR-6017 registered higher quantum of polyphenols than any other tea clones; however, both the clones registered on par values in terms of total polyphenols. As mentioned earlier, productive and drought tolerant tea clone, UPASI-9 recorded intermittent values. On the other hand, both UPASI-20 and TRI-2043 clones registered significantly lower values.

Considering the process methods, modified processing retained significantly higher amount of polyphenols than that of conventional method. Unlike the orthodox and CTC black teas, in white tea processing is very simple, devoid of cutting, rolling, fermentation and drying process. Hence the unoxidised bio-constituents are intact, as it was available in the fresh flush. It is often described that white tea processing steps are very few and unoxidised. However, in conventional process of white tea, it is slightly oxidised during prolonged withering (http://www.thespruce.com/whatiswhitetea766437 3/6).

Polyphenol content was significantly higher in the modified method of white tea processing. Higher polyphenol retention in the modified method may be attributed to the complete arrest of enzymatic oxidation in the handpicked buds. It has been reported that the percentage of total phenolics of fresh shoots of TRI-2043 was about 23% on dry matter basis and it was 21.2% in the processed Ceylon silver tips (Gunawardana *et al*., 2010). In the present study, white tea derived from processed white tea recorded 21.7%;, irrespective of the processing methods; the variation may be due to growing conditions, sampling time, processing method, etc. (Himangshu Deka *et al*., 2021).

Table 1. Total polyphenol content in white tea samples due to clonal variations and processing methods

Total polyphenols (%)			
Tea clone	White tea processing		Mean (Clone)
	Modified method	Conventional method	
UPASI-9	26.49	22.30	24.40
UPASI-20	24.74	19.20	21.97
TTL-2	29.49	22.60	26.05
CR-6017	30.38	22.80	26.59
TRI-2043	24.44	18.96	21.70
Mean (Process method)	27.11	21.17	-
Statistical significance: P = 0.05:			
	S.E.	C.D.	C.V. (%)
Processing method	0.65	1.27	8.19
Clones	0.82	1.59	-

As it was expected and catechins are integral part of polyphenols, distribution of catechins followed similar trend identical to the total polyphenols with minor deviations (Table 2). According to the factorial design statistical analysis, along with the quality tea clones (TTL-2 and CR-6017), popular drought resistant UPASI-9 also combined and formed a group containing higher catechin contents. All the three clones contained statistically on par values in terms of total catechins where it ranges between 15.52 and 16.01% irrespective of processing methods. Interestingly UPASI-20 registered intermittent values of catechins while TRI-2043 recorded lower values, irrespective of the processing methods. However, modified method of white tea processing edge over the conventional method of production of white tea and the values are statistically significant at five per cent level. Total catechins varied significantly among the tea clones, irrespective the processing methods. Even though tested tea clones were cultivated under identical climatic conditions and adopted unique agronomic practices, they exhibited variations in total catechin content substantiating their origin from different tea growing locations (Saravanan *et al*., 2005; Himangshu Deka *et al*., 2021).

Table 2. Total catechin content in white tea samples due to clonal variations and processing methods

Total catechins (%)			
Tea clone	White tea processing		Mean (Clone)
	Modified method	Conventional method	
UPASI-9	17.93	13.38	15.66
UPASI-20	14.74	10.28	12.51
TTL-2	18.69	13.33	16.01
CR-6017	19.01	12.03	15.52
TRI-2043	12.55	10.57	11.56
Mean (Process method)	12.55	10.57	-
Statistical significance: P = 0.05:			
	S.E.	C.D.	C.V. (%)
Processing method	0.38	0.75	5.27
Clones	0.46	0.89	-

Primarily, catechins are comprised of several fractions like simple catechin (+C), (-) epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC), and (-) epigallocatechin gallate (EGCG) (Saravanan *et al*., 2005). It has been reported that among the processed teas, white tea considered as abundant source of proanthocyanidins, phenolic acid derivatives and acylated glycosylated flavonols. Among the tea clones analysed, EGCG was the predominant catechin fraction and it was found higher in TTL-2 followed by UPASI-9 and CR-6017 (Table 3). The trend was not followed identically with respect to other catechin fractions. ECG and EGC were almost equally distributed followed by simple catechins and epicatechins. Retention of gallic acid in the processed white tea was abundant and results obtained from the present endeavour are substantiate the findings of Karori *et al*. (2007).

Table 3. Variations in Catechin fractions due to clones and processing method

Catechin fractions (%)	Tea clones					
	UPASI-9	UPASI-20	TTL-2	CR-6017	TRI-2043*	TRI-2043**
Gallic acid	0.80 ± 0.14	0.81 ± 0.01	0.80 ± 0.01	1.06 ± 0.09	1.02 ± 0.05	0.61 ± 0.05
EGC	2.33 ± 0.24	2.82 ± 0.11	2.61 ± 0.10	2.98 ± 0.20	2.08 ± 0.07	1.84 ± 0.06
(+) C	1.06 ± 0.17	1.04 ± 0.16	1.09 ± 0.07	0.99 ± 0.09	0.44 ± 0.06	0.22 ± 0.01
EC	0.87 ± 0.08	0.54 ± 0.05	0.69 ± 0.04	1.80 ± 0.12	0.35 ± 0.01	0.12 ± 0.01
ECGC	11.65 ± 0.23	9.28 ± 0.43	12.7 ± 0.15	11.41 ± 0.34	7.54 ± 0.24	6.36 ± 0.37
ECG	2.02 ± 0.01	1.06 ± 0.04	1.60 ± 0.17	1.84 ± 0.02	2.12 ± 0.22	1.83 ± 0.09
Total	17.93 ± 0.15	14.74 ± 0.79	18.69 ± 0.34	19.01 ± 0.09	12.55 ± 0.59	10.37 ± 0.35
White tea processing: * modified method; ** conventional method						

Individual catechin fractions are grouped as gallated and non-gallated catechins. Catechin fractions are also formed as trihydroxylated and dihydroxylated catechins on the basis of chemical structure/nature. Catechin index is an important biochemical marker, which is derived with values of catechins fractions (Owuor and Obanda, 2007). Considering the computed values of gallated catechins of white tea processed with different tea clones, TTL-2 registered higher values followed by UPASI-9, CR-6017, UPASI-20 and TRI-2043 (Table 4). However, CR-6017 recorded higher values with regard to non-gallated catechins, dihydroxilated- and trihydroxyated-catechins and catechin index. Except the dihydroxylated catechins, TRI-2043 occupied the last position among the clones with regard to catechin groups/catechin index. Values of catechin index ranged from 1.07 to 1.98. Interestingly, almost two-fold increase in the values of catechin index was observed in CR-6017 followed by other clones which is due to compositional variation in different catechin fractions.

Table 4. Compositional groupings of catechin fractions and catechin index of white tea derived from clonal teas

Catechin groups[@]	Tea clones					
	UPASI-9	UPASI-20	TTL-2	CR-6017	TRI-2043*	TRI-2043**
Gallated catechins	13.60	10.34	14.30	13.25	9.66	8.19
Non gallated catechins	3.20	3.36	3.30	4.78	2.43	1.96
Dihydroxylated catechins	2.89	1.60	2.29	3.64	2.47	1.95
Trihydroxylated catechins	13.98	12.10	15.31	14.39	9..62	8.20
Catechin index	1.43	1.51	1.43	1.98	1.17	1.07

White tea processing: * modified method; ** conventional method
[@]Gallated catechins: (EGCG + ECG); non-gallated catechins: (EC + EGC); dihydroxylated catechins (DHC): (EC + ECG); trihydroxylated catechins (THC): (EGC + EGCG); CI (catechin index): (DHC/THC)

As mentioned earlier, based on the catechin fractions and their groups served as an indicator in predicting the genetic diversity of tea clones (Jibu Thomas *et al*., 2008; Ashu Gulati *et al*., 2009). Though the present study is not intended to classify the tea clones on the basis of catechin fractions, the results substantiates the predominant role of catechins as a tool to classify the unknown tea germplasm. As stated earlier, CR-6016 and TTL-2 represents high grown quality clones which can be utilized for white tea processing followed by UPASI clones.

Conclusion

The study demonstrated that modified method of white tea processing can reduce time without affecting the quality attributes. In fact, polyphenols and catechin contents were significantly higher in modified method. Present study is not only aimed to reduce the white tea processing time but also to identify the alternate tea clones for white tea (speciality tea) preparation. The study provides finer details on total polyphenols, catechins and their fractions of white tea produced from certain elite cultivars of southern India. On the basis of compositional data on catechins groups, certain tea clones were identified as highly suitable for white manufacturing, particularly CR-6017 and TTL-2 besides UPASI clones on the basis of higher amount of catechins.

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