Synthesis of Value Added Tea Product by Enzymatic Treatment Employing FAR Derived Tannase

K.E. Nandini[1] and S. Krishna Sundari[2*]

[1,2]Biotechnology Department, Jaypee Institute of Information Technology, A-10, Sector-62, NOIDA, U P, India
E-mail: [1]nandini8874@gmail.com, [2]krishna.sundari@jiit.ac.in

Abstract—*Tea is the most important agriculture product with record high of 11.12 million tonnes production in India and registered global consumption of 800 million cups/day. There is a growing demand for healthy options of tea with improved quality. With such high consumption levels of tea, any research on synthesis of value added tea products would have ready acceptability. Current study is an attempt for enzymatic treatment of tea to improve its quality. Four tea samples namely green tea (GT), branded black tea (BT), loose tea of bigger grain size (LTB) and loose tea of smaller grain size (LTS) were procured from Delhi-NCR region and subjected to enzymatic treatment. In order to economize the process and also to limit addition of high cost ingredients we have used enzyme tannase produced from food and agricultural residues (FAR) through submerged fermentation. Native isolate Aspergillus carbonarius was used for fermenting the substrate and produce tannase extracellularly. Four concentrations of FAR derived tannase (20, 40, 60 and 80 U/100ml of tea extract) were used for the treatment of tea extract. Quality parameters tested were pH, total polyphenols (TPP), antioxidant property and total suspended solids (TSS). Enzymatic treatment has resulted in improvement of all parameters tested at all enzyme concentrations. However, best improvement was observed either at 40 or at 60U enzyme/100ml tea extract depending upon the tea sample used. Before enzymatic treatment, TPP was below the ISO (International standards for Tea) norms for all samples except for GT and post enzymatic treatment there was a significant improvement (8.5-fold increase) in LTS sample. Increase in anti-oxidant property was highest with BT (rise from 2.22 mg/ml to 9.12 mg/ml Trolox equivalent)which is an increment of 75%. Another significant value addition was in terms of tea cream reduction wherein a 30% reduction was obtained in TSS increasing its palatability and quality. From the results of the study we conclude that enzymatic treatment of tea using FAR derived tannase could prove to be of considerable value addition for tea products offering better health benefits.*

Keywords*: Value added tea, Enzymatic treatment, Health benefits of tea, Tannase, Tea polyphenols, Antioxidant property.*

1. INTRODUCTION

Next to water, tea is the cheapest and most popular beverage in the world. Also considered a poor man's beverage it is equally entertained across all strata in the society. Tea comprises of major components such as catechins and polyphenols, which have beneficial health improving qualities as well as nutritional value [1-2]. In fresh tea sample 20-30% of the dry weight contains catechins and 80% represents total polyphenol. Tea polyphenols contributes greatly to tea taste and nutrional value represented by tea phenolic acids, catechins, flavonoids and anthocyanins. The major catechins available in tea are (-)-epigallo catechin gallate (EGCG), (-)-epigallo catechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epicatechin (EC). These components are responsible for eliciting several health benefits including antimutagenic, antioxidant, anticarcinogenic properties. Antioxidant tea polyphenols offers both chemoprotective and therapeutic benefits in assisting cancer therapy. Apart from cancer, tea polyphenols were also reported to help in conditions related to cardiovascular disease, neurodegenerative, promotion of oral health, anti-hypertensive effect, controlling body weight, antibacterial activity, protection from ultraviolet rays, increasing bone mineral density, anti-fibrotic properties etc. Apart from these tea is also known for its nutritional value as it contains proteins and mineral salts [3-6].

The conventional process adopted for preparing instant tea involves hot water extraction of tea, then agitation along with low temperature, followed by centrifugation of tea cream. The tea cream thus separated contains major flavor components and is usually discarded. This results in considerable loss of the nutritional components [4, 7]. Alternative chemical method adopted for solubilizing tea cream is by means of treating it with sulfite and molecular oxygen together followed by an alkali treatment. Instant tea powder produced by chemical methods when reconstituted further as a hot beverage with milk would result in a dull blackish/ unpleasant coloration. Unpleasant coloration and tea cream formation during the processing of tea are due to coacervation of tea flavonoids, formation of hydrogen bond between caffeine and tea polyphenols. Compounds thus formed by hydrogen bonding and coacervation are poorly soluble [4]. To obtain

instant tea with good color, high solubility of nutritional component and good yield, it is necessary to eliminate such poorly soluble compounds without affecting the nutritional value of the tea in contrary to both conventional/chemical methods [4, 7-9]. In order to overcome the limitations of above methods in tea processing, enzymatic tea treatment would be most suitable. Enzymatic treatment makes it possible to obtain a cold water soluble tea retaining available aromatic compounds (essential nutrients) and appropriate color of tea.

Most widely used enzyme during tea processing for manufacture of instant tea/green tea is tannase. Tannase used in tea processing is commercially produced using costlier synthetic tannic acid medium. Tea processing thus becomes costlier as the cost of tannase production would also be included along with other manufacturing cost of tea. So the cost of instant tea/green tea available in the market becomes costlier and will be an expensive option for public consumption. To make the process economical, we need to focus on producing tannase using cost effective methods. This would be possible only if costlier synthetic tannic acid is replaced by cheaper alternatives as natural substrates for production of tannase. This would facilitate pricing of treatment a more economical range while offering high nutritional value and better health benefits.

Our study was focuses on production of tannase using easily available cheaper substrates such as food and agricultural residue (FAR) and successful application of tannase thus produced for the enzymatic treatment. Production of tannase on FAR through submerged fermentation (SMF) was administered using native fungal isolate *Aspergillus carbonarius*. Enzymatic treatment using FAR derived tannase in order to obtain tea product with better health benefits by enhancing tea polyphenols and antioxidant property of tea products.

2. MATERIALS AND METHODS

2.1 Preparation of tea extract: Four tea samples namely green tea (GT), branded black tea (BT), loose tea of bigger grain size (LTB) and smaller grain size (LTS) were procured from Delhi-NCR region. One gram of above tea samples were mixed with 100 ml boiling water in a 250 ml beaker individually. Beakers were then placed in a water bath maintained at 85 ºC for 40 minutes as described in protocol by Lu and Chen [10]. Tea extract was cooled to room temperature, filtered through whatmann No. 1 filter paper for further enzymatic treatment.

2.2 Preparation of crude enzyme-tannase for enzymatic tea treatment: FAR combination for production of tannase used in the study was pomegranate peel powder and spent tea powder in 1:1 ratio. Fungal culture *Asperigillus carbonarius* used in this study for submerged fermentation for tannase production was maintained on Czapek-Dox agar supplemented with 2 % tannic acid. 4 g of FAR combination was mixed with 50 ml of modified salt media (MSM) which contained only the mineral salts (K_2HPO_4 1.52, KCl 0.52, $MgSO_4$ 0.52, $NaNO_3$ 3.0, $FeSO_4$ and $ZnSO_4$ 0.10in g/L) and devoid of any carbon source. FAR medium (FARM) prepared was autoclaved at 15 lb, 120 °C for 20 min. FARM media was inoculated with 0.5 mm dia mycelial disc of *Asperigillus carbonarius* cut from 7 day old fungal colony. Inoculated FARM media was incubated at 30 °C and 150 rpm for a time interval of 48 h. Post fermentation culture media was centrifuged. The supernatant thus obtained was filtered using 0.2 micron filter to remove the spores of the fungal culture. Tannase activity of the filtrate obtained was 80 U/ml and one ml of this filtrate is diluted to obtain four different concentrations tannase using millipore water.

2.3 Enzymatic treatment: Enzyme solution thus diluted to obtain four different tannase concentrations of 20, 40, 60 and 80 U/ml considered as treatment T1, T2, T3 and T4 respectively was used for enzymatic tea treatment. 1ml of tannase solution was added to 100ml of four tea extract individually and incubated at room temperature for 2h.

2.4 Pre and post treatment analysis of tea extract:Tea extract of GT, BT, LTB and LTS, pre and post treatment were analysed for pH, DPPH and H2O2 scavenging activity, TSS, TPP and tea cream.

The pH of tea extract pre and post enzymatic treatment was measured using calibrated pH meter (Orion,).

Total polyphenols were estimated using Folin–Ciocalteu's reagent as described by Hong and co-workers [11]. The reaction mixture includes 0.79 ml of distilled water, 0.01 ml of sample, and 0.05 ml of Folin–Ciocalteu reagent. After exactly 1 min, 0.15 ml of 20% sodium carbonate will be added to the mixture, followed by incubation for 2h at room temperature in darkness. The absorbance will be measured at 750 nm and total polyphenol content will be calculated with GA as standard.

Total suspended solids of tea extract were measured using dried tea extract (hot air oven at 103 ºC until a consistent dry weight of sample was obtained). TSS was expressed in g/l.

Tea cream solids that are insoluble in cold water were estimated after chilling the tea extract for 16 h at 5 ºC and centrifugation at 5600g for 20 min to remove the insolubles from the extract [12]. The difference between the estimated soluble solids in primary tea extract and its supernatant was represented as tea cream solids after cooling.

Antioxidant property: DPPH radical scavenging and H_2O_2 scavenging effect were measured as per the method discussed by Lu and Chen (2008) [13]. DPPH scavenging activity was expressed in mg/ml trolox equivalent. The percentage improvement of H_2O_2 scavenging effect was calculated as per the equation given below.

Scavenging effect (%) = $[(A_B-A_S)/A_S] \times 100$

Where A_B is the absorbance of the blank and A_S is the enzymatic treated tea sample.

3. RESULT

Enzymatic treatment of different tea samples

Enzymatic treatment of green tea showed improvement in its quality with all treatment concentrations of tannase as compared to control but maximum enhancement was observed with T2 i.e. 40 U/100 ml tea extract. Though there is significant increase in TPP beyond T2 concentration, it was not reflected equally well with other parameters tested. The increase in DPPH, H_2O_2 scavenging effect and TPP was 50.6%, 56.1% and 101.5%, respectively and reduction in pH, TSS and tea cream was 19%, 35.4% and 37.5% respectively (Table. 1). Branded tea quality has been found to be higher with tannase treatment T4 in terms of TPP enhancement (7.49 times), reduction in tea cream (56.5%) and TSS (50%). However, increase in antioxidant property was not remarkable i.e. only 47%. The enhancement in antioxidant property was 310% with treatment T2 which was much higher as compared to TPP improvement (3.16 times against 7.49 times at T4), reduction in TSS (26.3% against 50%) and tea cream (28.3% against 56.5%). Hence the optimum concentration of tannase enzyme for treatment of BT was considered as T2 where an optimal increase in all parameters studied was observed (Table. 2). Similar results were observed with the loose tea where the optimum concentration of tannase for better enhancement with all parameter was T2. Though the increase in TPP was above 4.98 times at T4 concentration as against 2.27 times with T2 concentration, the antioxidant property found to be lower than the control at T4 tannase concentration (Table. 3). DPPH scavenging activity was found to decrease with the increase in tannase enzyme concentration used for the treatment. Beyond the tannase treatment level T2, decrease in DPPH scavenging activity was about 50%. Further at T2 the enhancement observed in other parameters was, increase in TPP by 1.58 times and 24% enhancement in H_2O_2 scavenging effect with the reduction in TSS to 26.1%, tea cream to 23% with reduction in DPPH was only up to 13% which could be considered negligible as compared to 50% reduction at T4 tannase concentration (Table. 4).

4. DISCUSSION

The results of the present study support use of enzyme for improved beneficial quality of tea products. Enhancement in antioxidant property after enzymatic treatment could have been due to enhancement in free radical scavenging as reported in the literature [13]. It has been earlier reported that enzymatic treatment results in increased gallic acid content which improves scavenging activity/behavior in the media/substrate that contains superoxide anions/hydroxyl/peroxyl radicals [2, 13-14]. Further it is also found that the treatment of tea with enzyme enhances the natural level of epicatechin and gallic acid leading to formation of epitheaflavic acid which is responsible for bright reddish color of tea [15]. Reduction in tea cream could be due to reduction in TSS and also increase in natural gallic acid concentration in tea product because of tannase treatment which in turn increases the solubility level by increasing the tea cream solubility [12]. Marginal decrease in tea pH could be due to increase concentration of polyphenol compounds.

5. CONCLUSION

Present study demonstrated that the enzymatic treatment of tea using tannase enhances the recovery of polyphenols, increase the antioxidative property of the tea by maintaining a good balance of tea quality. Study also reveals the application of enzymes as a safe and organic method of tea processing that circumvents the problems of conventional and chemical processing. Tannase enzyme being capable of hydrolyzing the tea as a substrate has significant important role to play in enhancing the antioxidant property of various tea samples. However, the problem of costly production of tannase, the crucial enzyme which needs synthetic tannic acid for its own production has been solved to some extent by using the FAR derived tannase for enzymatic treatment of tea in this study. Hence the study demonstrates that there is a scope of possible commercial application of FAR derived tannase for enzymatic treatment in order to increase the health benefits of tea.

Table 1: Properties of green tea extract pre and post enzymatic treatment

Sample	pH	TSS (g/l)	DPPH scavenging (mg/ml trolox equivalent)	H2O2 scavenging (%)	TPP (mg/g)	Tea cream (mg/100 ml)
Control	5.54	48	5.95	36	224.08	0.96
T1	4.48	42	8.99	58.57	322.65	0.8
T2	4.48	31	8.96	56.19	451.57	0.6
T3	4.41	30	8.98	48.76	629.04	0.6
T4	4.28	27	9.07	37.81	709.88	0.54

Table 2: Properties of branded tea extract pre and post enzymatic treatment

Sample	pH	TSS (g/l)	DPPH scavenging (mg/ml trolox equivalent)	H2O2 scavenging (%)	TPP (mg/g)	Tea cream (mg/100 ml)
Control	5.04	38	2.22	19.81	69.92	0.92
T1	4.66	34	8.41	43.14	198.35	0.66
T2	4.52	28	9.12	36.19	291.09	0.66
T3	4.48	22	3.24	32.57	576.35	0.50
T4	4.29	19	3.26	24.10	593.59	0.40

Table 3: Properties of Loose tea extract of bigger grain size pre and post enzymatic treatment

Sample	pH	TSS (g/l)	DPPH scavenging (mg/ml trolox equivalent)	H2O2 scavenging (%)	TPP (mg/g)	Tea cream (mg/100 ml)
Control	5.12	44	9.40	18.38	100.75	0.88
T1	4.91	33	10.01	37.24	176.98	0.70
T2	4.5	32	9.66	29.14	329.93	0.68
T3	4.37	28	9.09	22.76	445.01	0.60
T4	4.28	24	5.21	17.14	602.82	0.42

Table 4: Pre and post enzymatic treatment properties of Loose tea extract of smaller grain size

Sample	pH	TSS (g/l)	DPPH scavenging (mg/ml trolox equivalent)	H2O2 scavenging (%)	TPP (mg/g)	Tea cream (mg/100 ml)
Control	5.49	46	9.38	14.95	133.04	0.76
T1	4.73	35	8.68	53.62	193.74	0.61
T2	4.46	34	8.14	39.71	343.53	0.58
T3	4.34	29	4.66	35.14	481.43	0.50
T4	4.31	27	4.66	26.76	582.42	0.40

6. ACKNOWLEDGEMENTS

The study is an integral part of postdoctoral research fellowship funded by the Department of Biotechnology (DBT), Govt. of India, India. The authors gratefully acknowledge the financial support by DBT and thank Biotechnology Department, JIIT, NOIDA for providing laboratory and infrastructure facility.

REFERENCES

[1] Anesini, C., Ferraro, G. E., and Filip, R., "Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina", *Journal of Agricultural Food Chemistry, 56,* 2008, pp. 9225–9229.

[2] Macedo, J. A., Battestin, V., Ribeiro, M. L., and Macedo, G. A., "Increasing the antioxidant power of tea extracts by biotransformation of polyphenols", *Food Chemistry*, 126, 2011, pp. 491-497.

[3] Hicks, A., "Review of global tea production and the impact on industry of the asian economic situation", Commodity Market Review, FAO Regional Office for Asia and the Pacific Bangkok, Thailand, June, 1998, pp. 1-8.

[4] Beniwal, V., Kumar, A., Sharma, J., and Chhokar, V., "Recent Advances in Industrial Application of Tannases: A Review", *Recent Patents on Biotechnology*, 7, 2013, pp. 228-233.

[5] Yang, C. S., Lambert, J. D., and Sang, S., "Antioxidative and anti-carcinogenic activities of tea polyphenols", *Archieves of Toxicology,* 83, 1, January 2009, pp. 1-17.

[6] Bian, M., Skidmore, A. K., Schlerf, M., Liu, Y., and Wang, T., "Estimating biochemical parameters of tea (*Camellia sinensis* (L.) using hyperspectral techniques", *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, Volume XXXIX-B8, 2012, XXII ISPRS Congress*, August-September 2012, Melbourne, Australia.

[7] Nagalakshmi, S., Jayalakshmi, R., Seshadri, R., "Approaches to decreaming of black tea infusions by solvent decaffeination and tannase treatment", *Journal of Science and Food Agriculture*, 22, 1985, pp. 198-201.

[8] Sanderson, G. W., and Coggon, P., "Green tea conversion using tannase and natural tea enzymes", US 3812266, 1974.

[9] Coggon, P., Graham, H, N., and Sanderson, G. W., "Cold water soluble tea", BP1380135, 1975.

[10] Lu, M. J., and Chen, C., "Enzymatic tannase treatment of green tea increases *in vitro* inhibitory activity against N-nitrosation of dimethylamine", *Process Biochemistry*, 42, 2007, pp. 1285-1290.

[11] Hong, Y. H., Jung, E. Y., Noh, D. O., and Suh, H. J., "Physiological effects of formulation containing tannase-converted green tea extract on skin care: physical stability, collagenase, elastase, and tyrosinase activities", *Integrative Medicine Research*, 3, 2014, pp. 25–33.

[12] Chandini, S. K., Rao, J. L., Gowthaman, M. K., Haware, D. J., and Subramanian, R., "Enzymatic treatment to improve the quality of black tea extracts", *Food Chemistry*, 127, 2011, pp. 1039-1045.

[13] Lu, M. J., and Chen, C., "Enzymatic modification by tannase increases the antioxidant activity of green tea ", *Food Research International*, 41, 2008, pp. 130-137.

[14] Kanwar, J., Taskeen, M., Mohammad, I., Huo, C., Chan, T. H., and Dou, Q. P., "Recent advances on tea polyphenols", *Frontier Bioscience*, 4, March 2012, pp. 111–131.

[15] Natarajan, K., "Tannase: A tool for instantaneous tea", *Current Biotica*, 3, 1, 2009, pp. 96-103.