Simultaneous Determination of Catechins, L-Theanine, Theaflavins (Flavanoids & Antioxidants) From Green Tea Infusion And Its Influence of Water Quality on Nutritional Characteristics Using HPLC

J. Sudharson[1], S. Yuvaraj[2], G. Tamilarasan[3], M. Raja Periya Nayagam[4], C. Aravindh[5]
[1, 3, 4, 5] Dept of Chemical Engineering
[2]Assistant professor, Dept of Chemical Engineering
[1, 2, 3, 4, 5] Adhiyamaan College of Engineering, Dr. MGR Nagar, Hosur, Tamil Nadu - 635130, India.

Abstract- *Influence of water quality on the green tea extraction and their constituents was studied. The concentration of the catechins, L-theanine and theaflavins was higher in the water containing lower total dissolved solids, conductivity and pH. The total phenolic content and FRAP values was also determined respectively. The extractability difference was explained using the mass transfer equation. The whole process was carried out in the High-performance liquid chromatography (HPLC). By the study it is recommended to use the water having lower TDS, conductivity and pH for hot infusions and RTD beverages.*

Keywords- Water quality, Total dissolved solids, Total phenolic content, pH, HPLC and RTD

I. INTRODUCTION

Green tea is made from leaves and buds of *Camellia sinensis*. Most widely consumed ready to drink beverages is green tea. It originated at China and its production and manufacture has spread to East and Southeast Asia. The tea is planted above the sea level because higher the height above the sea level, higher tea properties. Tea has divided into three types they are green tea, oolong tea and black tea. The content of total polyphenol of the green tea depends upon the growing conditions, climate and the leaf processing method. The main polyphenols found in green tea include catechins, theanine, theaflavins and flavanoids. The flavanoids are classified into three types known as kaempferol, quercetin and myricetin. Catechins are the natural antioxidents that helps to prevent cell damage and provides many health benefits. Green tea has higher phenolic content concentrations and the activities of antioxidant than the black tea. Infusion is the most popular method of preparing green tea. Green tea infusion can be prepared by infusing some grams of dried tea leaves in hot water and leaved for some time so that the extracts can be transferred to the water and cooled to room temperature. The filtrate is known as the infusion and this can be processed through various processes so that we can determine the constituents of the green tea. Water quality is one of major thing that impact on the quality of the extraction. We should be aware of the water characteristics, that is total dissolved solids (TDS), conductivity and pH of the water used for infusion.

II. MATERIALS AND METHODS

Green tea

Green tea (*Camellia sinensis*) has got from Indfrag Biosciences Private Limited, Bengaluru, India. The tea particle size averages about 1.2 mm, found by using sieve analysis.

Chemicals, Solvents, reagents and standards

The standards of Catechin, Epicatechin, Epicatechin gallate, Epigallocatechin, EGCG, gallic acid, theaflavins and glacial acetic acid was bought from Sigma Aldrich Chemicals, Bengaluru, India. Acetonitrile, formic acid, sodium carbonate, methanol, and water. These are of high pressure liquid chromatography (HPLC) grade which is obtained from Nawal Analytical Laboratories, Hosur, India. The Folin-Ciocalteu reagent was from KK International (Kolkata), other reagents were used of analytical grade and bought from Nawal Analytical Laboratories.

Water types used and their sources

The raw water is the tap water supplied to the laboratory (TP). Reverse osmosis water (RO) was the water purifier output. Processed and packaged drinking water (PD) through various stages of purification was bought from the local market. Soft water (SF) was obtained by water softener

while treating the raw water. The final product output of the Milli-Q water purifier was ultra-pure water (UP).

Sample preparation

The infusion was prepared by brewing 2.5 g of made tea in 250ml of water at temperature of 98-100°C about 3 mins. The obtained infusion by this process was filtered using a tea strainer, temperature of the infusion of was decreased to ~25°C by using a cold water bath. The cooled infusion was then centrifuged at 3000 rpm for about 15 min in order remove the suspended particles.

Measurement of quality of water and infusion

Conductivity, total dissolved solids (TDS) and pH was measured with the help of portable conductivity meter.

TABLE 1: Quality of water samples

SI. NO	Water type	Conductivity (µS)	TDS (ppm)	pH
1	TP	629.4	312	7.24
2	ST	677	335	7.91
3	RO	25.4	12.6	6.05
4	PD	11.4	5.21	6.75
5	UP	1.27	0.63	6.11

TABLE 2: Quality of infusion made by different types of water

SI. NO	Water type	Conductivity (µS)	TDS (ppm)	pH
1	TP	749	372	6.92
2	ST	886	439	7.15
3	RO	223	113	5.12
4	PD	229	115	5.32
5	UP	211	107	5.55

Mineral content

The mineral content of the water samples used was determined using Atomic Absorption Spectrometer, which is having a single furnace atomizer.

Evaluation of total soluble solids of tea

The total soluble tea solids (TSS) of infusion was estimated using hot air oven by drying the infusion at 105°C for 4 hours. Total soluble solids of tea is the difference between the mass of TSS in water and that infusion respectively.

Evaluation of catechin contents

The types of catechins that is EC, ECG, EGC and EGCG, of the green tea was determined by using the standards specified according to the ISO. The process was conducted out by using a HPLC system that consists of a pump, Ultra Violet detector (model:2489) and control module of pump. The phenyl-hexyl column, 250 × 4.6 mm with 5 µm particles was used with the HPLC system. The detection wavelength was 285 nm and the column temperature was maintained at 38°C and the experiment was carried out. The data processing was done using ChromNAV 2.0 software, which is provided with the HPLC system. In order to avoid any changes in the catechin content , samples were freshly drawn from prepared infusion that is stored in a RT at 4°C and stabilized.

Evaluation of L-theanine

The L-theanine content of the green tea is determined by using a HPLC system that contains a c-18 column, 300 × 4.6 mm with 5 µm particles. The analysis was carried out with a modification in the elution program that is the rate was set at for 0 – 5th min, 2% acetonitrile and 98.5% ultrapure water; 5 – 14th min, the % of acetonitrile was gradually increased to 50%; for 14–30th min the acetonitrile and ultrapure water maintained at 50% each; for 30–45th min, the acetonitrile was decreased gradually to 2%. The concentration of acetonitrile and ultrapure water was keep on same till 50th min.

Estimation of theaflavins

The theaflavins (TF, TF-3-G, TF-3'-G and TF-3, 3'-G) contents of the green tea was quantified using the HPLC system with a C-18 column was used. The dimensions of the column is 100 × 4.6 mm with 2 µm. The mobile phase A and B are 10% acetic acid and acetonitrile to ethyl acetate in a ratio of 8:1. The analysis shows that the 75% of the mobile phase A at 3 mins provides better result in separation of four theaflavin monomers.

Total phenolic content

By using the folin-ciocalteau procedure, the total phenol content of the infusion was determined, with some changes. The sample of infusion is 2 ml and 10% of sodium carbonate solution is 8 ml, were added a reagent of folin-ciocalteau is 10 ml. Let mixture is react in dark room with

room temperature of 25°C about 90 min. The absorbance were study at 650nm.

Estimation of ferric reducing antioxidant power (FRAP)

It was accomplished by measuring the change in absorbance at 585 nm. FRAP made up by mixing with acetate buffer is 300 mM, pH is 3.7, TPTZ is 10 mM and $FeCl_3 6H_2O$ (20 mM) to make ratio of 10:1:1. 330 µL of FRAP at 35°C was added to 150 ml of prepared green tea infusion immediately

III. RESULTS AND DISCUSSION

Influence of water on mineral concentration of the infusion

Since the water samples (TP, ST, RO, PD and UP) was analyzed in the atomic absorption spectrometer the results were noted on the individual mineral. Then the mineral content of infusion was quantified the results shows that potassium (≤51.0 mg/L); copper (≤0.0224mg/L); magnesium (≤4.528 mg/L); manganese (≤0.526mg/L) and zinc (≤0.081mg/L). These minerals where leached to the infusion. By seeing the analytics we can conclude that water quality has no effect in the leaching of minerals.

Table 3: Mineral content of water samples

CHEMICALS	TP (mg)	ST (mg)	RO (mg)	PD (mg)	UP (mg)
Calcium	50.85	0.345	0.92	0.184	0.195
Sodium	29.4	40.32	1.26	0.794	0.183
Potassium	1.36	0.563	0.054	0.052	N.D
Magnesium	16.92	0.372	0.184	0.051	0.011
Manganese	0.001	N.D	N.D	N.D	N.D
Zinc	0.104	N.D	N.D	N.D	N.D
Copper	N.D	N.D	N.D	N.D	N.D

TP - Tap water; ST - Soft water; RO - Reverse osmosis water; PD - Packaged drinking water; UP - Ultrapure water.

Table 4: Mineral content of the infusion

CHEMICALS	TP (mg)	ST (mg)	RO (mg)	PD (mg)	UP (mg)
Calcium	47.72	0.438	0.542	0.247	0.143
Sodium	30.92	43.12	1.53	1.17	0.156
Potassium	55.48	57.13	47.32	50.67	44.97
Magnesium	18.72	1.837	0.687	0.824	0.537
Manganese	0.572	0.546	0.516	0.523	0.478
Zinc	0.156	0.079	0.084	0.054	0.032
Copper	0.017	0.023	0.027	0.021	0.024

For abbreviations see in table 3.

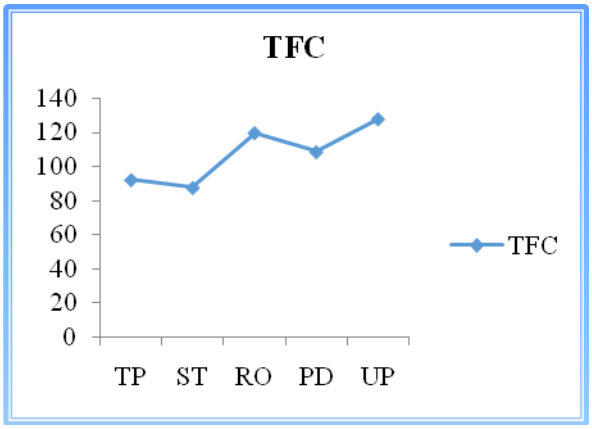
Dissolved tea solids, total catechins, total polyphenols and L-theanine concentration

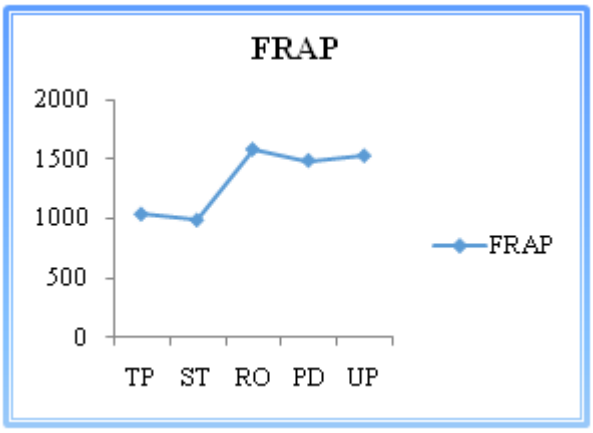
The extractability of the total polyphenols, catechins (EC, EGCG, EGC and ECG), tea solids and L-theanine was notably higher in the RO, PD and UP, when compared to that of ST and TP infusions and the total polyphenol content in the RO, PD and UP is greater than the ST and TP infusions of green tea. The total polyphenols and theanine concentrations in the green tea infusion prepared using the RO water which is notably higher when compared to the infusion prepared using other infusions. Here we getting the results like extractability depend upon the ionic strength of the water that is used for the infusion preparation. The extraction of the total catechin and total polyphenols content was also follows the same concept only. Lower the pH was in advantageous for the extractability of the epi-structured catechins. It is found that the total polyphenol content is lower than the total catechins concentration in the major cases. Hence we conclude that the extractability of the total polyphenols and total catechins are higher in the UP, PD and RO than the ST and TP.

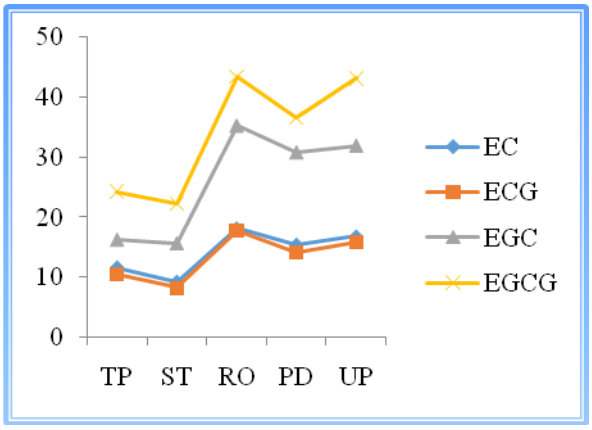
The difference in concentration reduces, by the driving force, the concentration of the dissolved components in water is increases. So that the mass transfer rate is reduced, which causes lower extractability in water containing high soluble solids (TP and ST), RO, PD and UP has higher extractability

TABLE 5: The concentration of reactions on tea extracts

PARTICULARS	FRAP mg/g	TSS %	TPC mg/g	TFC mg/g	EC mg/g	ECG mg/g	EGC mg/g	EGCG mg/g
WATER								
TP	1034.4	0.094	29.81	92.31	11.62	10.51	16.25	24.28
ST	984.8	0.091	25.62	87.68	9.21	8.25	15.65	22.33
RO	1574.3	0.110	49.5	119.74	18.15	17.75	35.35	43.45
PD	1481.4	0.107	40.75	108.62	15.42	14.17	30.9	36.6
UP	1521.6	0.109	47.46	127.86	16.79	15.84	32.03	43.13

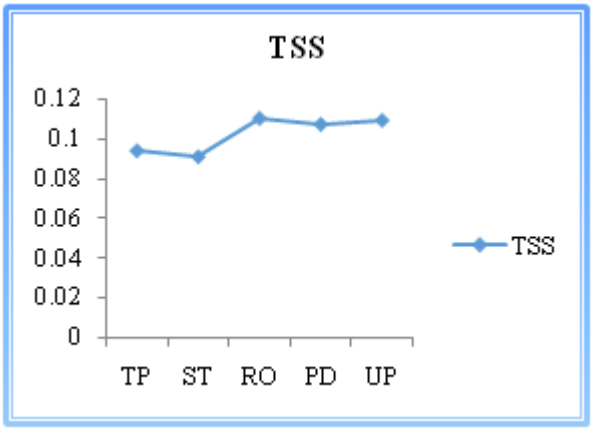


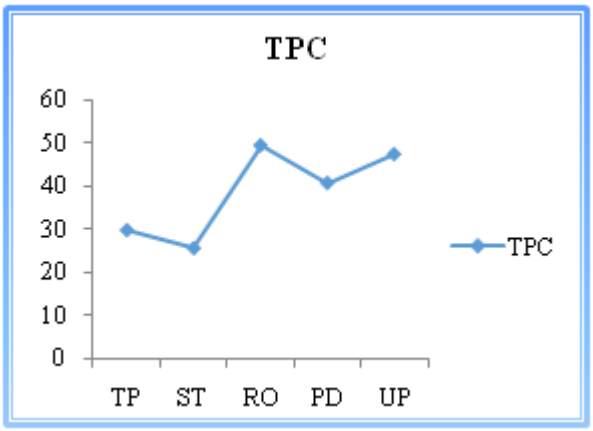




Total theaflavin content

Theaflavins (TF) and its derivatives are collectively known as theaflavins. These are kinds of antioxidant polyphenols. The theaflavins are mainly present in the black tea only but some small amount of theaflavins can be able present in the green tea also we can determine it by using analytical method of analysis. Here acetic acid is used as the mobile phase A of the extraction of





theaflavin at the concentration of 10% of dilution. If we increase the concentration of the acetic acid it will

decrease the separation degree of the theaflavins. Then the mobile phase B is acetonitrile and ethyl acetate at the ratio of 8:1 respectively. The flow rate is maintained at 1.5 ml/min. It is found traceable amount of theaflavins is extracted in the RO and UP water samples. There is no theaflavins detected in the in the infusion prepared by using the TP, ST and PD water. The analytical method was observed in the HPLC system with C-18 column. Results may vary when varying the temperature, time of infusion and using different solvent and HPLC columns for the extraction.

Total phenolic content

The phenolic compounds the major source of antioxidants found in the green tea. The difference in extraction was observed in the infusions, the infusion made with TP gives the lesser phenolic content followed by ST, PD, RO and UP. The ion of the water samples is the major criteria the transfer of the phenolic content from the tea leaves to the water. Temperature and was kept at constant for all the infusions. Now we can understand that the temperature and the time of infusion is the dominant criteria for the extraction of the phenolic compounds. Therefore we determine that UP, RO and PD water is suitable for maximum extraction the total phenolic content. But it is also important to consider the brewing temperature of the infusion. Different temperatures can result in different values of the phenolic content.

IV. CONCLUSION

Finally, the study concludes that the water quality influence of green tea having low flavonoids, antioxidants, total theaflavin content and total phenolic content should be recommended for hot drinks and beverages.

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