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Effect of brewing temperature, tea types and particle size on infusion of tea components

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Abstract

Infusion profiles, partitioning property and spectrophotometric correlations for different components present in tea have been discussed in the present work. The infusion profiles of four different marketed tea brands were studied over 50-100°C. The results obtained from the studies show the direct relationship of infusion profiles with brewing temperature and different particle size fractions. The HPLC analysis of brew shows the epicatechin gallate (ECG) and catechin gallate (CG) to be fast eluting compared to other components studied. The partition constants of major catechins and methyl xanthines between swollen tea granules and aqueous solution have been determined. The partition constants (K) of all components range between 0.23-0.82 g/mL over 60-80°C. The correlation between the most commonly used methods in routine tea brew analysis namely, delivered polyphenol content (DPP), UV spectrophotometry and HPLC have been developed.

Introduction

Tea *(Camellia sinensis)* is the most widely consumed beverage in the world. It has been studied extensively to determine the brewing kinetics (Spiro and Siddique, 1981a, 1981b; Spiro and Jago, 1982; Price and Spiro, 1985a; Spiro *et al*., 1992; Bronner and Beecher, 1998; Fernández *et al*., 2000; Merken and Beecher, 2000; Wang *et al.*, 2000; Sakakibara *et al*., 2003). The major phytochemicals of tea are polyphenols including catechins and methyl xanthines. The catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), catechin gallate (CG) and methyl xanthines contain caffeine, theobromine (TB), theophylline (TP) (Harbowy and Balentine, 1997; Lee *et al*., 2014). Catechins constitute about 3-10% of black tea (figures expressed on dry matter basis (Graham, 1992; Khokhar and Magnusdottir, 2002). The organoleptic and sensory attributes of different tea brews are related to different components present in it. Depending on the extent of the solubilized components, the color and taste of the brew vary (Harbowy and Balentine, 1997; Lee and Chambers, 2009; Chaturvedula and Prakash, 2011). Also, commercial development of tea as a health drink or nutraceutical formulation will require knowledge of the extent of bioactives present in them.

In order to clearly state the objectives, a comparison of previous literatures related to present research work is shown in Table 1. Development of various analytical methods for determination of polyphenols, catechins, and caffeine in different tea brands and at various experimental conditions were studied in the past using spectrophotometry (Spigno and De Faveri, 2009; Suteerapataranon *et al*., 2009) and chromatographic analysis (Terada *et al.*, 1987; Goto *et al*., 1996; Zhu *et al.*, 1997; Khokhar and Magnusdottir, 2002; Liang *et al*., 2003; Cheong *et al*., 2005; Sharma *et al.*, 2005; Quan *et al*., 2006; Suteerapataranon *et al.*, 2009; El-Shahawi *et al*., 2012; Rahim *et al.,* 2014). Development and optimization of chromatographic methods with variation in stationary phases and elution conditions has been studied in depth for determination of tea catechins (Dalluge *et al*., 1998). Selective extraction of EGC and EGCG-enriched fractions from tea at different conditions of brewing temperature and brewing duration has also been explored using numerous methods (Labbé *et al*., 2006). Polyphenol and xanthine estimation using gravimetric methods have been attempted to establish the contributions of product and preparation variables on the total soluble solids in brew (Astill *et al*., 2001; Liang *et al.,* 2005). Determination of partition constant of some of the components present in tea and coffee like caffeine, theaflavins and thearubigins has been studied in the past (Spiro and Siddique, 1981a; Price and Spiro,

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Table 1. Comparative literature review of the previous work

Tea Type	TWR	T (°C)	Brewing time (min)	Methods employed				Correlation amongst various methods	Brand variation	PS variation	*K*
				UV (nm)	DPP/TPP	DSS/TSS	HPLC (nm)				
Black tea leaf (Spiro and Siddique, 1981b)	-	79.5 or 94	20	380/273	-	-	-	-	-	-	TF, TR, Caffeine
Black tea leaf (Price and Spiro, 1985a)	-	80	30	625	-	-	275	-	Yes	-	TF, Caffeine
Green, Oolong and black tea (Terada *et al.*, 1987)	1:80	100	3	-	-	-	280	-	-	-	-
Green & black tea (Spiro *et al.*, 1992)	1:50	60-90	30-60	-	-	-	275	-	-	Yes	-
Green & black tea (Shao *et al.*, 1995)	1:40	100	10	-	-	-	280, 380, 450	-	-	-	-
Green tea (Goto *et al.*, 1996)	1:200[#]	Room temp.	40	-	-	-	231	-	Yes	-	-
Kenyan arabica coffee beans (Spiro *et al.*, 1997)	1:100	25.5, 80	-	273	-	-	273	-	-	-	Caffeine
Green tea (Zhu *et al.*, 1997)	1:35[@]	80	-	-	-	-	280	-	-	-	-
Green tea (Dalluge *et al.*, 1998)	-	80	10	-	-	-	210	-	-	-	-
Black & green tea (Wang *et al.*, 2000)	1:50	-	5	-	-	-	210/280	-	Yes	-	-
Black & green tea (Astill *et al.*, 2001)	-	-	-	-	TPP	TSS	278	-	Yes	Yes	-
Green, Oolong Black & fruit teas (Khokhar and Magnusdottir, 2002)	1: 100**	60-100	5	-	TPP	-	278	-	Yes	-	-
Black tea bags (Lian and Astill, 2002)	1:75	90	5.8	445	-	TSS	-	-	-	-	-
Black tea (Liang *et al.*, 2003)	1:50	-	10	-	-	-	280	Sensory-HPLC	Yes	-	-
Green tea (Cheong *et al.*, 2005)	1:50	60, 80, 100	240	-	-	-	281	-	-	-	-
Black & green tea (Sharma *et al.*, 2005)	1:25*	-	-	-	-	-	210/275	-	Yes	-	-
Green tea (Labbé *et al.*, 2006)	1:50	50-90	80	-	-	-	210	-	-	-	-
Green tea (Quan *et al.*, 2006)	-	80	-	-	TPP	-	230	-	-	-	-
Black tea bag (Spigno and De Faveri, 2009)	1:100	-	-	280	TPP	-	-	-	-	-	-
Green & Oolong tea (Suteerapataranon *et al.*, 2009)	1:100	80-100	60	273	-	-	-	-	Yes	Yes	-
Green tea (El-Shahawi *et al.*, 2012)	1:400	90	30	-	-	-	205	-	Yes	-	-
Green, Black, Oolong teas (Rahim *et al.*, 2014)	1:50[##]	-	6	-	-	-	280	-	-	-	-
Black Tea (Present work)	**1:50**	**50-100**	**15**	**272/445**	**DPP, TPP**	**DSS**	**272**	**DPP-UV-HPLC**	**Yes**	**Yes**	**Catechins-methyl xanthines**

T: Temperature; TWR: Tea water/solvent ratio; PS: Particle size; *K*: Partition constant; [#]Acetonitrile-water (1:1, v/v); [@]Water, 80% ethanol, [**]Water, 80% methanol or 70% ethanol; [*]Acetonitrile, water, methanol, aqueous methanol, acetone; [$]100 mL water and 99% ethanol; [##]Acetonitrile, water and methanol combinations

1985b; Spiro and Chong, 1997).

None of the literatures cited in Table 1 explains the correlation between UV spectrophotometry and polyphenol estimation using various methods. Also, simultaneous estimation of partitioning property of major catechins and methyl xanthines present in tea has never been discussed till date. Hence, the objectives with novel aspects of the present work are: (i) Interrelationship of different tea brew analyses like dissolved solids (DSS) and polyphenol content (DPP) with UV spectrophotometry; (ii) Interrelationship between HPLC and UV spectrophotometric methods for total content determination, (iii) Simultaneous determination of partition coefficients of various components like gallic acid (GA), EC, EGC, ECG, EGCG, CG, caffeine, TB and TP, (iv) Determination

of fast and slow eluting components and (v) Effect of particle size on the release of the various tea components. The developed interrelationships were established for commercially available tea brands with different particle sizes and over a wide range of temperatures. Thus these correlations will be beneficial in convenient, fast and economic analysis of tea brews. The deduction of fast-slow eluting components and partition constants will aid in predicting the quality of tea brews.

Materials and methods

Commercially available four black CTC teas (S1, S2, S3, S4) were procured from the local market. Of these, S1 and S2 are from same manufacturer, with S2 being stronger in sensory perceptions than that of S1. For estimating the effect of particle size on infusion kinetics, different particle fractions of tea brands were used. The general method for preparing and determining the particle size for various fractions was used (Farakte *et al*., 2016). Five different particle sizes were used: retained on 850 µm (F1), between 850 and 710 µm (F2), between 710 and 500 µm (F3), between 500 and 212 µm (F4) and passing through 212 µm (fines). The particle sizes of F1-F4 were determined using ImageJ™ software (Farakte *et al*., 2016). The total number of particles analyzed for each fraction was about 500.

Pure standard of GA, EC, (-) EGC, (-) ECG, (-) EGCG, (-) CG, caffeine, TB and TP were purchased from Sigma Aldrich chemicals, Bangalore. Folin-Ciocalteu reagent and anhydrous sodium carbonate were purchased from S. D. Fine chemicals, Mumbai. Acetonitrile and ortho-phosphoric acid (85%) of HPLC grades were used. Milli-Q (Millipore, Bedford, MA, USA) treated de-ionized (DI) water was used throughout the studies.

Tea brewing kinetics protocol

2 g of tea and 100 mL DI water was used for brewing experiments. Tea brewing was performed at 50-100°C as described by Farakte *et al*. (2016). The effect of temperature, tea types, measurements at different wavelengths were statistically analyzed using two - way analysis of variance (ANOVA).

GA is a base molecule for most of tea polyphenols (Harbowy and Balentine, 1997). A 100 mg/L stock solution of GA was prepared in volumetric flask. This stock solution was further used for preparing working standard solutions of 1-100 mg/L. These solutions were scanned from 200-800 nm using UV-visible spectrophotometer (Varian CARY 50 Conc, Agilent) to determine λ_{max}. A calibration curve was plotted and the linear equation thus obtained was used for determining gallic acid equivalents (GAE) in tea brews. The GAE% was calculated on the basis of 2 g tea used and corrected for cumulative GAE lost due to previous samples (Farakte *et al.*, 2016).

Selection of absorption wavelength

Different absorption wavelengths have been tried for analysis of tea catechins, varying from 205 to 280 nm (Shao *et al*., 1995; Horie *et al.*, 1997). Due to the various compounds present in tea, the absorbance of tea brews is found to be higher near two wavelengths i.e. near 205 and 275 nm. Therefore, the samples were diluted 100 times (100X) and scanned from 200-400 nm using UV-visible spectrophotometer to determine the λ_{max} against water as blank. The UV spectrum of 100X diluted sample shows the λ_{max} to be 272 nm. Hence, 272 nm was selected for as analytical wavelength. For confirmation of λ_{max}, λ_{max} of all standards was calculated using Woodward Fieser rules for dienes for (Pavia *et al.*, 2001). The major components absorb near 270-275 nm. The calculated λ_{max} of major gallates (EGCG, EGC, CG, ECG) and methyl xanthines differ because of the no. of polar groupings (-OH) present in the structure of gallates. Most abundantly present theaflavins and thearubigins are responsible for golden yellow and reddish brown color of the brew. These constituents absorb in the wavelength range 440–460 nm (Lian and Astill, 2002). Hence, the samples were diluted 20 times (20X) and the absorbance was measured at 445 nm as an indicator for brew color.

Stability studies

The tea brew is a mixture of various phytochemicals viz. catechins, methyl xanthines, amino acids, etc (Harbowy and Balentine, 1997). Hence, it becomes crucial to know about the stability of samples to be stored and analyzed. 100X diluted samples were stored at 4°C (in refrigerator) and room temperature for 24 h and their absorbance were compared. The samples were found to be stable under refrigeration for 24 h and hence all samples were stored in refrigerator for further analysis.

Delivered polyphenol (DPP) content

The amount of delivered polyphenol in brew was determined using Folin-Ciocalteu method (ISO, 2005). GA was used as standard and the calibration was performed over concentration range 1-100 mg/L. The DPP was expressed as GAE (mg/mL) using Folin-Ciocalteu method. The absorbance of these solutions was measured at 765 nm using spectrophotometer. The DPP% is determined as mg/g of tea input used.

Dissolved soluble solids (DSS)

DSS is a measure of the total amount of solubilized tea components (Harbowy and Balentine, 1997). To determine the DSS, 2 g of tea was brewed in 100 mL of water. After 0.5 min, the experiment was stopped and brew was filtered immediately. 20 mL of this filtrate was dried in tared petri dish in hot-air oven at 80-90°C till constant weight was obtained. DSS was calculated as weight% (wt%) of 2 g tea input. The experiments were carried out for different brewing durations of 0.5, 1, 3, 5 and 15 min at 60 and 80°C. Also, the absorbance of each brew was measured at 272 nm to check the relationship between absorbance and DSS%. The experiments were performed in duplicate for validation.

HPLC analysis

Tea brews of different experiments were analyzed for the components present in them. The system used was Agilent 1260 infinity series model. A phenomenex Prodigy-ODS-2® column (150 × 4.6 mm) fitted with a phenomenex SecurityGuard® column C18 ODS (4 x 3.0 mm) was used for characterization of tea components.

Previous HPLC method developed utilized 5% acetonitrile (eluant A) and 25% acetonitrile (eluant B) in phosphate buffer (Khokhar and Magnusdottir, 2002). This method was modified by preparing eluants in water. The pH of both the eluants was adjusted to 2.4 using ortho-phosphoric acid. The gradient elution programming was employed as: 0-5 min, 15% B; 5-20 min, linear gradient 15-80% B; 20-23 min, 80% B; 23-25 min, 15% B. The flow rate was 1 mL/min and injection volume was 20 µL. Stock solutions of pure standards of GA, EC, EGC, ECG, EGCG, CG, caffeine, TB and TP (1 mg/mL in water) were used for calibration. The detection wavelength is 272 nm and the peaks were integrated using the chemstation software. Caffeine was used as reference peak to calculate internal response factors of all the other standards. Calibration of caffeine from 1-20 mg/L was performed for estimation of caffeine concentration in tea brew. Total concentration released (mg/mL) was calculated by adding the concentration of each component released in the brew. Variation in the area of each component peak was determined by repeating brewing experiments in triplicate for S2 at 70°C. Standard deviation and % error for the average value of area were calculated for all components for each experiment for validation. Two - way ANOVA was utilized for statistical analysis.

Partition constant determination

Equilibrium brewing experiments were performed for S1 unground particles at 60-80°C for determination of partition constants (Farakte *et al*., 2016). Un-ground 1.2 mm and 0.41 mm particles of S1 were chosen to study the effect of particle size on equilibrium behavior of various components. In a set of equilibrium brewing experiment, minimum three extractions were carried out at different ratio of tea weight to water. 50 mL of water was kept constant and weight of tea was varied (0.5, 1, 1.5, 2 g). The brewing time was kept as 15 min. 1 mL of brew was withdrawn and filtered to exclude the particles in the brew. Remaining brew was filtered and volume of brew obtained and weight of wet tea granules were measured. The partition constant (K) of different constituents present in the brew were calculated by equation (1), given by Spiro and Siddique (1981a).

$$K = \frac{Concentration\ in\ brew}{Concentration\ in\ tea\ particles} = \frac{C_\infty}{(wx_0 - (V - wV_n)C_\infty)/w`} \quad (1)$$

K = Partition constant considering water absorption in tea particle

C_∞ = Concentration of component at equilibrium (mg/mL)

w = Weight of tea used for brewing (g)

V = Volume of water used for brewing (mL)

x_0 = Initial amount of component present per g of tea (mg/g)

V_n = Volume of water absorbed per g of tea (mL/g)

w` = Weight of tea ghost after complete brewing (g)

Equation (1) can be rearranged as

$$\frac{1}{C_\infty} = \frac{V}{wx_0} + \frac{1}{x_0}\left(\frac{w`}{wK} - V_n\right) = \frac{V}{wx_0} + \frac{1}{x_0K`} \quad ... (2)$$

where $$\left(\frac{w`}{wK} - V_n\right) = \frac{1}{K`} \quad ... (3)$$

K` is apparent/fictional partition constant without considering water absorption in tea. A plot of $1/C_\infty$ against 1/w should produce a straight line. Its slope will give x0 and a combination of slope and intercept will estimate the partition constants K' and K.

Statistical analysis

The effect of temperature, tea components concentration data using HPLC, partition constants determination and readsorption constant determination were subjected to analysis of variance (ANOVA) using MS Excel 2007 (Microsoft Office 2007, USA). Linear regression was utilized for establishing the relationship between DPP-UV-HPLC.

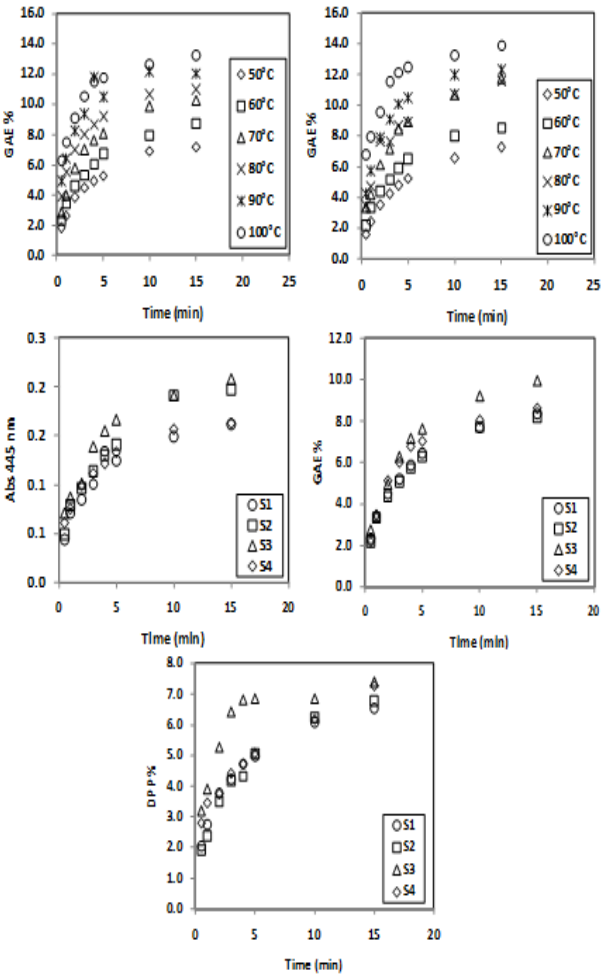

Figure 1. (i) Effect of temperature on GAE% for (a) S1 (b) S2 over 50-100°C; (ii) Comparison of different brands for (a) absorbance at 445 nm; (b) GAE%; (c) DPP% at 60°C

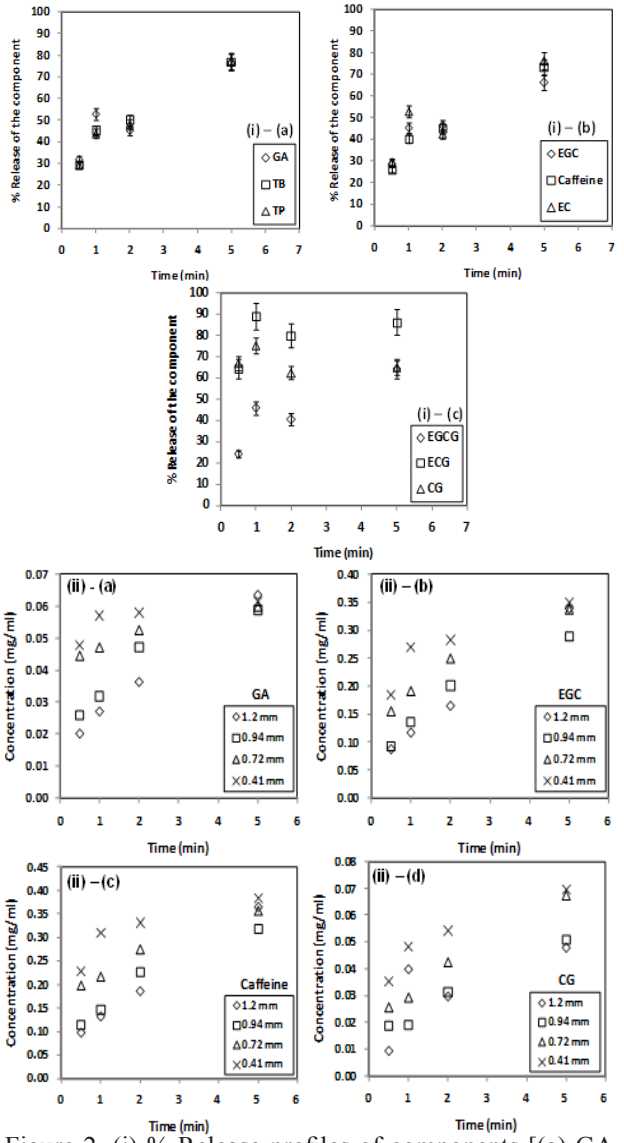

Figure 2. (i) % Release profiles of components [(a) GA, TB, TP; (b) EGC, Caffeine, EC; (c) EGCG, ECG, CG)] from S2 at 60°C up to 5 min (ii) Effect of particle size on release profiles of components [(a) GA, (b) EGC, (c) Caffeine, (d) CG] from S1 at 60°C up to 5 min

Results and Discussion

Tea infusion kinetics

The effect of temperature on infusion profile of S1 and S2 was studied over the temperature range 50-100°C. The calibration of GA is performed at 272 and 765 nm for determination of GAE% and DPP%. The calibration is found to be linear at both wavelengths over concentrations 1-30 mg/L (272 nm) and 1-100 mg/L (765 nm). The coefficient of regression (R^2) is 0.994 and 0.997 for 272 and 765 nm respectively. These calibration curves give two linear equations which were used to calculate the GAE% and DPP% at 272 and 765 nm respectively.

Figure 1(i) (a-b) depicts the effect of temperature (50-100°C) on GAE% at 272 nm for S1 and S2 respectively with respect to time. It can be observed from the figures that an increase in temperature from 50-100°C results an increase in the infusion rate. This trend is observed in both brands. The rate is very fast up to 3 min and then gradually slows down up to 15 min for both. It is observed from the results of statistical analysis that tea catechins and xanthines solubilization are highly dependent on the brewing temperature ($p<0.001$).

Tea brew color is one of the many parameters affected by the infusion of soluble components in brew. In the present work, the brew color is determined by measuring the absorbance of 20X diluted sample at 445 nm. In order to compare the effect of various brands on measured parameters, the infusion profiles of S1-S4 are compared at 60°C [Figure 1(ii) (a-c)]. The comparison of absorbance at 445 nm for different brands is shown in Figure 1(ii-a). Of the four brands studied here, S3 shows relatively highest absorbance while S1 shows lowest absorbance up to 15 min. The difference in their absorbance is more pronounced at 4 min onwards.

Table 2(a). Effect of tea type and temperature on tea components infusion up to 5 min (mg/2g of tea input) in S2 tea

Groups	Components	Effect of tea type	Effect of temperature	
		S1_60°C	S2_60°C	S2_80°C
Methyl xanthines	Caffeine	22.59	23.69	37.74
	TB	1.43	3.23	4.62
	TP	0.03	0.07	0.23
Gallates	EGC	17.58	12.24	11.89
	ECG	3.36	2.26	2.46
	EC	2.90	5.63	12.21
	EGCG	3.34	1.39	5.63
	CG	0.63	0.28	1.56
	GA	4.13	3.40	5.97
Total concentration		**55.99**	**52.19**	**82.30**
Total catechin concentration		**31.94**	**25.20**	**39.70**
Total methyl xanthines concentration		**24.05**	**26.99**	**42.59**

This difference in the absorbance at 445 nm is due to the extent of water soluble components in the brew. It was also found that the absorbance at 445 nm increases with an increase in temperature from 60-80°C for a particular tea type. Figure 1(ii-b) shows the effect of different brands on GAE%. There was no significant change observed up to 2 min for GAE% of these brands. After 2 min, the GAE% for S3 increases when compared with S1, S2 and S4. At the end of 15 min, the GAE% for S1, S2, S3, S4 are 8.4, 8.2, 10.0 and 8.6 respectively at 60°C. Hence, it can be concluded that the GAE% differs for different brands of teas. Another important parameter computed is the DPP%. DPP% is calculated on the basis of 2 g of tea used. Figure 1(ii-c) shows the effect of different brands on DPP% at 60°C. The rate of infusion in terms of DPP% is highest for S3, compared to other brands. There is no significant difference observed in the DPP% of S1 and S2 for entire duration of 15 min. It is observed from the figure that S3 shows drastic increase in infusion rate up to 5 min. At the end of 15 min, the DPP% of S1, S2, S3, S4 are 6.5, 6.8, 7.4 and 7.3% respectively at 60°C.

The DSS% for S1 and S2 at 60°C for 15 min is 28.2 and 27.2% respectively. The error in the DSS% values is found to be ±5%. At 80°C, DSS% values for S1 and S2 are 27.3 and 29.6% respectively at 15 min. Hence, the DSS% values vary with respect to brewing temperature.

HPLC analysis

A mixture of five catechins, GA, caffeine, TB and TP were successfully separated by gradient RP-HPLC method. The HPLC separation is achieved in 23 min, with a total run time of 35 min. Calibration curve of caffeine shows linear behavior with R2 as 0.997. The error in measuring the concentration of components using HPLC analysis is found to be ±7%.

The estimation of fast and slow eluting components in the brew will help us to understand the elution kinetics at different tea components. This has not been discussed in the previous literature. Hence, the rate of elution of different components present in tea is compared in terms of % of total concentration released with respect to time. Concentration released at 15 min was used to calculate % total concentration released. Figure 2(i) (a-c) illustrates the % of total components released from S2 at 60°C in 5 min. From Figure 2(i-a), it can be observed that % release profile of GA, TB and TP from S2 is approximately same up to 5 min. About 45% was released at the end of 1 min for these components. At the end of 5 min, the release is found to be 77%. As shown in Figure 2(i-b), similar release profile of EGC, caffeine and EC was observed. EC shows marginally higher release with 53% at 1 min when compared with EGC and caffeine. All the components depicted in Figure 2(i) (a-b) collectively release average 47% up to 1 min. Figure 2(i-c) depicts the % release profile of EGCG, ECG and CG. The % release profile of ECG is highest (89%) followed by CG (75%) at the end of 1 min. EGCG shows 46% release, similar to components shown in Figure 2(i) (a-b). Therefore, ECG and CG can be regarded as fast eluting with 89% and 75% release respectively in barely 1 min. Other compounds namely GA, TB, TP, EGC, caffeine, EC and EGCG can be groups as slow eluting components having average 47% release at the end of 1 min.

The level of catechins is determined in S1 and S2 at 60°C and 80°C up to 5 min. Effect of tea types and temperatures on tea components concentration was determined. The effect of tea types and temperature on their concentration is depicted in Table 2(a). The concentration is calculated in mg per 2 g of tea used. Two-way ANOVA is utilized for statistical analysis. The p-value for the kinetics of catechins

Table 2(b). Partition constants (K, K') and initial concentrations of tea constituents (x_o) in S1 tea at different temperatures (60-80°C) and for different particle fractions (1.21 and 0.41 mm)

Components		Effect of particle size						Effect of Temperatures						Mean x_0 (mg/g)	Mean x_0 (wt%)
		1.21 mm (60°C)			0.41 mm (60°C)			70°C			80°C				
		K (g/mL)	K' (g/mL)	x_0 (mg/g)	K (g/mL)	K' (g/mL)	x_0 (mg/g)	K (g/mL)	K' (g/mL)	x_0 (mg/g)	K (g/mL)	K' (g/mL)	x_0 (mg/g)		
Methyl xanthines	Caffeine	0.41	0.24	31.25	1.08	1.68	26.31	0.67	0.51	32.28	0.76	0.65	33.50	32.34	3.23
	TB	0.59	0.41	1.56	0.82	0.76	1.69	0.82	0.77	1.62	0.40	0.23	1.85	1.67	0.17
	TP	0.72	0.59	0.16	1.17	2.40	0.19	0.74	0.62	0.15	0.74	0.62	0.19	0.17	0.02
Gallates	EGC	0.43	0.25	13.42	0.21	0.10	12.47	0.66	0.49	16.21	0.75	0.64	14.85	14.83	1.48
	ECG	0.28	0.10	1.25	0.19	0.09	1.48	0.25	0.12	2.79	0.23	0.11	2.69	2.24	0.22
	EC	0.28	0.14	9.73	0.66	0.50	24.37	0.50	0.32	22.11	0.74	0.62	21.56	17.80	1.78
	EGCG	0.28	0.14	5.81	0.44	0.27	4.74	0.43	0.25	4.54	0.52	0.34	6.56	5.64	0.56
	CG	0.23	0.12	4.51	0.33	0.18	6.41	0.72	0.59	4.21	0.79	0.70	5.33	4.68	0.47
	GA	0.43	0.26	3.67	0.19	0.09	8.65	0.46	0.28	4.26	0.56	0.38	6.80	4.91	0.49
													Total x_0	84.28	8.43

and xanthines solubilization is 0.00001. With respect to tea type effects in Table 2(a), it is noted that the total concentration of components released up to 5 min is 55.99 mg (2.80%) and 52.19 mg (2.61%) for 2 g of S1 and S2 respectively at 60°C. The level of catechins in S1 (31.94 mg) is higher than that of methyl xanthines (25.20 mg). Conversely, in case of S2, the concentration of methyl xanthines released (26.99 mg) is slightly higher than that of catechins (25.20 mg). The Concentration of TP released is very less in the final brew at 5 min (S1-0.03 mg; S2-0.07 mg). The contribution of caffeine released in the final brew at 5 min is highest (1.1% in both teas) followed by EGC (0.6-0.8%). This observation is in good agreement with the previous work (Harbowy and Balentine, 1997; Fernández *et al*., 2000). With reference to the wt% delivered to brew from granules, 8.43% soluble constituents elute out from the granules. This is in good agreement with the values reported previously (Graham, 1992; Harbowy and Balentine, 1997; Astill *et al*., 2001; Khokhar and Magnusdottir, 2002).

The temperature of extraction plays an important role for diffusion of water into tea particle and solubilization of tea components in leaf matrix. An increase in the solubilization of components and thereby diffusion to the outside bulk is observed with temperature increments in the present work. To study the effect of temperature on infusion of different components, the experiments were carried for S2 at 60 and 80°C [Table 2(a)]. An increase in the infusion of components is observed with increase in temperature from 60 to 80°C. The concentration of GA released at 80°C is 1.8 times of 60°C. While for compounds like TB, caffeine, the concentration released at 80°C is 1.4-1.6 times of 60°C. For TP, the concentration released at 80°C is 3.2 times of 60°C. The highest increment in concentration at 80°C is noted for CG (5.6 times) followed by EGCG (4.0 times). It is identified that the infusion profile of EGC and ECG is approximately similar at both temperatures up to 5 min. It was observed that the concentrations of gallated moieties eluted are higher at 80°C than at 60°C. Hence, the temperature of extraction is a significant parameter in the infusion profile of different components. The concentration of total components released is in the range of 52.19 - 82.30 mg for 2 g of S2 over 60-80°C. The caffeine contributes 1.2 and 1.9% of total concentration at 60 and 80°C respectively. The HPLC analysis data for infusion profiles of different teas at different temperatures is validated statistically by p-value of 0.041.

Effect of Particle size

The particle size of different fractions prepared from S1 and S2 is measured by ImageJTM software. The details of their particle sizes for S1 (unground-1.21 mm) and S2 (unground-2.2 mm) are 0.92 mm (F2), 0.72 mm (F3), 0.41 mm (F4-S1) and 0.36 mm (F4-S2). The effect of different particle size on the infusion of some of the components of S1 at 60°C is shown in Figure 2(ii) (a-d) up to 5 min. The infusion profile is shown for GA (a), EGC (b), caffeine (c) and CG (d) in Figure 2(ii). From the figures, it can be observed that as the particle size is reduced from 1.21 mm to 0.41 mm, the infusion profile for all the components has increased in the same order. A steep increase in the infusion profile up to 1 min and then comparatively slower infusion till 5 min is observed for all components. At 5 min, the difference in the infusion of different fractions is observed to be minimal for majority of components (except CG). The highest infusion is observed for

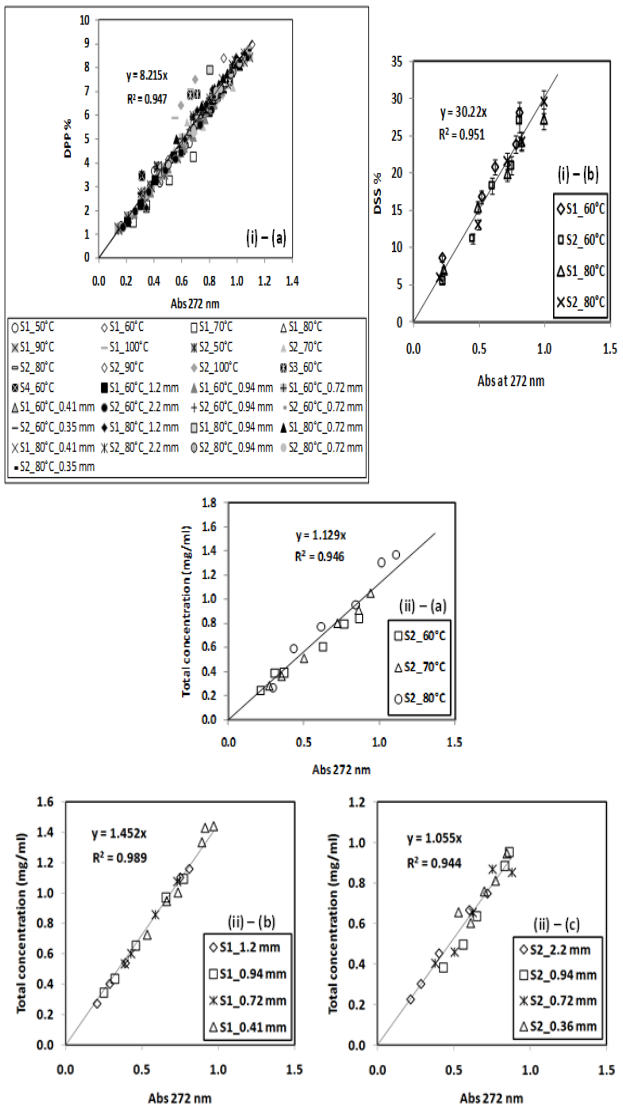

Figure 3. (i) UV correlations between absorbance at 272 nm and (a) DPP% for S1, S2 over different particle fractions and temperatures; (b) DSS% for S1, S2 at 60 and 80°C; (ii) Correlations between HPLC and UV absorbance at 272 nm for (a) different temperatures (60-80°C) for S2; (b) different particle fractions of S1 at 60°C (1.21-0.41 mm) ; (c) different particle fractions of S2 at 60°C (2.21-0.36 mm)

0.41 mm particles followed by 0.72 mm > 0.94 mm > 1.21 mm. Higher infusion of smaller particles is due to the increase in particle surface area because of reduction in particle size. An increase in infusion profile of major components with a decrease in particle size is also observed for S2 in 5 min at 60°C. The total concentration of components released in S2 brew at 60°C is highest for smallest particles i.e. 0.36 mm. Hence, smaller particle size accounts for higher infusion release of components for S1 and S2 at 60°C. These observations are in good agreement with the values reported previously (Spiro and Selwood, 1984; Price and Spiro, 1985a; Astill *et al.*, 2001).

Equilibrium brewing study

Partition constant determination (K, K')

It is a well known fact that partition constants vary with respect to temperatures and tea types. Hence, the effect of different temperature and two tea types on partition constants were investigated. Equation (2) shows that $1/C_\infty$ should vary linearly with 1/w. Such linearity is found for all of the nine components studied. The intercepts and slopes of these plots are computed by least square regression. The values of partition constant between swollen leaf (K) or leaf without water (K') and aqueous solution and concentrations of initial constituents (x_0) present in unground 1.21 mm S1 granules, over 60-80°C are summarized in Table 2(b). The effect of particle size on partition constants of different components was studied using unground (1.21 mm) and ground (0.41 mm) particles of S1 at 60°C using different tea:water ratio. In case of 0.41 mm particles, the K values for all components are found to be higher than that of coarser particles [Table 2(b)]. In case of EGC, the K value decreases slightly with decrease in particle size. Highest K value is observed for TP (1.17 g/mL) followed by caffeine (1.08 g/mL) when compared with its coarser fraction. All these observations are in correlation with the higher infusion profiles observed.

Concerning different temperatures, it can be seen that the all the values of K are in the range of 0.398-0.819 and 0.100-0.785 g/mL for methyl xanthines and gallates respectively over 60-80°C for unground particles of S1. The values of partition constants are observed to be increasing with increase in temperature. Higher K values are indicative of higher infusion of tea components in water as per equation (2). This is noticed for all of the components (except ECG). For ECG, the values of K are observed to be reducing marginally. The highest K value is observed for TP as 0.72-0.74 g/mL over 60-80°C. The values of *K* remain fairly constant for TP over 60-80°C. The partition constants listed in Table 2(b) have been reported for the first time for these constituents.

Concentration of constituents (x_o)

The x_o value of the components is calculated directly from equation (2). With increase in temperature, the x_o values are only slightly increasing [Table 2(b)]. However, this increase is less significant when compared with mean x_o. This is evident from the statistical p-value as 0.967 (>0.05). Higher p-value than 0.05 indicates that there is no significant difference in the initial amount present in tea (x_o) at various temperatures when compared with average values. It is possible that these components

are present in relatively inaccessible sites in the granules and so becomes fully extractable only at a higher temperature (Spiro and Price, 1987). It can be seen from Table 2(b) that x_o value is highest for caffeine (26.31 mg/g) followed by EC (24.37 mg/g) for smaller particles of 0.41 mm. Highest increase in x_o values, with a reduction in particle size from 1.21 mm to 0.41 mm is observed for EC (9.73 to 24.27 mg/g) followed by GA and caffeine (increase by 4.98 mg/g) at 60°C.

Relationships between different measurement methods

The absorbance at 272 nm is correlated with DSS%, DPP% and total concentration by HPLC (mg/mL) to establish a relationship between them. Figure 3(i-a) portrays the comparison of absorbance at 272 nm versus DPP% for S1 and S2 over 50-100°C, their different particle fractions, S3 and S4. It is observed that a good linear correlation is obtained between absorbance at 272 nm and DPP% for different teas at different temperatures (R^2 = 0.947). This is evident by linear regression analysis ($p<0.05$). Thus, absorbance at 272 nm can be considered as a quantitative measure of DPP% for different teas at different temperatures.

Another important and similar observation is made from the plot of absorbance at 272 nm v/s DSS%. The DSS% of S1 and S2 at 60 and 80°C is compared with absorbance at 272 nm [Figure 3(i-b)]. The figure shows linear relationship along all the points passing through origin (R^2 = 0.951). This is further statistically validated using linear regression ($p<0.05$). Thus, DSS% of different teas can be predicted using the correlation obtained from absorbance at 272 nm at respective temperatures and DSS% comparison. These correlations obtained will be useful for faster and time-saving determination of DSS% and DPP%, as compared to laborious methods for DSS and DPP determination using gravimetric and colorimetric methods respectively.

A similar methodology is explored for establishing a relationship between absorbance 272 nm and total concentration by HPLC (mg/mL). Unground and different particle fractions of S2 are compared at different temperatures. Figure 3(ii-a) depicts the relationship for S2 unground particles over 60-80°C. All the points exhibit linear relationship passing through origin with $R^2 = 0.946$. Similar observation is noted for particle fractions of S1 and S2 with R^2 value as 0.989 and 0.944 respectively [Figure 3(ii) (b-c)]. The slopes of these three straight lines are different, indicating that these relationships are specific for a particular tea brand and particle size. Hence, simple spectrophotometric method can be used to determine DPP%, DSS% and total concentration of components eluted, by substituting longer protocols for Folin-Ciocalteu, DSS and HPLC analysis.

Conclusion

The quality of infusion depends upon various parameters affecting tea infusion kinetics. These parameters include variation in brewing temperature, tea brands and their particle sizes. We have reported the effect of these parameters on various measurements of tea brew analysis i.e. DSS, DPP, GAE estimation using UV and concentration of tea components using HPLC. We observed that the infusion profile enhances with increase in brewing temperature and reduction in particle sizes. Tea with smallest particle fraction had the highest infusion profile. The infusion profile was found to be different for different brands. This can be attributed to variation in manufacturing and processing methods for different brands. These studies help in better understanding of tea infusion profiles and eventually benefit the consumer protocols of brewing tea. It has been possible to divide the studied tea components including catechins in two groups, fast eluting and slow eluting components using HPLC. Once the elution order of tea components is understood, prediction of sensory profiles of tea brews and selective extraction of these components is possible. We have determined the partition constants of nine major components present in tea, using HPLC method. The partition constants of some of tea components have been discussed for the first time to the best of our knowledge. With partition constants in hand, it is possible to predict the solubility and content of various components present in tea granules and thus the brew quality. We have also presented the first ever developed correlations between spectrophotometric analysis and DPP, DSS, HPLC measurements. From the industrial point of view, this suggests that simple UV-visible absorbance can substitute the conventional time-consuming measurements of DPP, DSS and total concentration of components measured using HPLC. These correlations will be beneficial in convenient, fast and economic analysis of tea brews.

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