Appendix A. Supplementary Materials

Quality characteristics of infusion and health consequences: A comparative study between orthodox and CTC green teas

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Supporting Information

1. Preparation of the calibration standard

Caffeine standard was used for quantification of individual catechins and caffeine. A caffeine standard stock solution of 1000 μg mL^{-1} was prepared. This stock solution was diluted to obtain standard solutions in the concentration range 5-25 μg mL^{-1}. These standard solutions were injected in the UPLC. A calibration curve was constructed using the peak areas against the respective concentrations. The regression equation of the calibration curve was: y=0.307x-0.927, with R^2=0.999. Slope (S) and standard deviation (δ) of the response were determined for the calibration curve. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 and 10 times of δ/S, respectively as described by Fernando and Soysa.[1] The LOD, LOQ, and recovery for caffeine were 0.50 μg mL^{-1}, 1.52 μg mL^{-1}, and 98.73%, respectively.

2. Determination of total polyphenol contents

The total polyphenol contents were determined according to the methods described by the International Standard Organization.[2] Briefly, 0.20 g of ground tea leaf sample was extracted by 5 mL 70% methanol at 70 °C in water bath for 10 min and allowed to settle down. The supernatant extract was transferred to a 10 ml volumetric flask. The process was repeated for one more time. The final volume was made up to 10 mL by adding 70% methanol. 1 mL of this extract was diluted to 100 mL with water in a volumetric flask. 1 mL of the diluted extract was transferred into a test tube and 5 mL 10% (v/v)

Folin-Ciocalteu reagent was added to it with vigorous stirring. After 3 min, 4 mL of 7.5% (w/v) sodium carbonate solution was added to the reaction mixture and mixed in a vortex. The reaction mixture was allowed to stand at room temperature for 60 minutes. The absorbance of the resultant mixture was measured at 765 nm in the UV-Vis spectrophotometer. A sample blank using water instead of tea extract was also measured. The measurement was done against a gallic acid calibration curve prepared using concentrations in the range from 10 to 50 μg mL^{-1} ($y=0.012x-0.046$, $R^2=0.999$).

3. Determination of catechin and caffeine contents

Catechin and caffeine contents were determined using International Standard Organization method.[3] The extraction was done as described in the determination of total polyphenols to a 10 mL volumetric flask. 1 mL of the resultant extract was diluted with stabilizing agent to a 5 mL volumetric flask. The stabilizing agent was prepared by using ascorbic acid (500 μg mL^{-1}), EDTA (500 μg mL^{-1}), and acetonitrile (25% v/v) in water. The diluted extract was filtered through 0.45μm syringe filters before quantitative determination using UPLC. The column was eluted using two solvent system consisting of 2% (v/v) acetic acid, 9% (v/v) acetonitrile in water (A) and 80% (v/v) acetonitrile in water (B). A gradient elution was set as 100% mobile phase consisted of A for 10 min followed by a linear gradient to 68% of A and 32% of B over 15 min and held at this composition for 10 min. The flow rate was 1 mL min^{-1}. The peaks were identified by comparing with catechins and caffeine standard peak. The quantitative determination of individual catechins and caffeine were done by using relative response factors of catechins with respect to caffeine as described in ISO14502-2:2005.[3]

4. Determination of theanine contents

Theanine contents were determined using the method described by Too *et al*.[4] Briefly, 1.00 g of finely ground tea sample was extracted with 100 mL boiling water in a hot plate with magnetic stirrer for 5 min. The extract was filtered and the volume was made up to 100 mL in a volumetric flask by adding water. The solution was filtered through a 0.45 μm membrane before chromatographic analysis. Theanine content was quantified using UPLC by injecting 20 μL of the filtrate. The column was eluted with 100% acetonitrile for 22 min. After completion of the run, the column was cleaned by eluting with 100% water before next run. The theanine content was determined by comparing with a standard calibration curve. The calibration curve was prepared by using theanine standard solution in the concentration range from 10 to 150 μg mL^{-1}. The regression equation obtained was $y=0.1131x+0.4561$with $R^2=0.999$.

5. Determination of water extract

Water extracts of the tea samples were determined by using the method described in ISO 9768: 1994.[5] Briefly, 2.00 g of tea sample was taken in a 500 mL conical flask and 200 mL hot distilled water

was poured into it. The mixture was refluxed for 1 hour with occasional rotation. The mixture was filtered immediately after reflux through a previously weighed sintered crucible. The flask was repeatedly washed out with hot distilled water for transferring the entire insoluble residue into the crucible. The residue was washed with 200 mL hot distilled water under suction, followed by drying in a hot air oven at 103±2º C for 16 hrs. Finally the weight of the residue with the crucible, after cooling in desiccators, was noted. The water extract was calculated from the difference in weight of tea sample and insoluble residue.

6. Transfer rate of bioactive compounds

The contents of total polyphenol, catechins, caffeine, theanine and water extract from cup of tea infusion were also determined. The infusions were prepared by brewing 2.00 g green tea in 150 mL boiling distilled water for 3 min and then filtered. Total polyphenol, catechins, caffeine and theanine contents of the infusions were quantified by using the same method as described in previous sections. The water extract for the infusion was determined with the residue after filtration using the method as described in previous section, with the only exception that washing of the residue was avoided here.

From the data, the transfer rate of these individual components in infusion with respect to the content in tea was calculated by using the following equation

$$Transferrate(\%) = \frac{Contentininfusion}{Contentintea} \times 100$$

7. Determination of sodium, potassium, fluoride and chloride

Sodium and potassium in infusion were quantified using a flame photometer at a wavelength of 589.0 and 766.5nm, respectively.[6] Quantitative determination of fluoride and chloride was performed using Ion Selective Electrode (ISE) meter.[7,8] Samples were pre-treated with total ionic strength adjustment buffer (TISAB) and 5M $NaNO_3$ solution as per manufacturer's guidelines. In brief, to every 9 mL sample, 1 mL TISAB were added with uniform stirring before measuring the final fluoride concentration by immersing the electrode in the sample solution. For chloride determination, samples were pre-treated with 5M $NaNO_3$ solution at 1:50 reagent to sample ratio.

8. Statistical analysis

Analysis of variance (ANOVA) was performed by using SPSS software version 17.00 (SPSS Inc., Chicago, IL). Tukey's multiple comparison test was used to get the differences between means and the differences were considered significant at $p \leq 0.05$ and $p \leq 0.01$. For each sample, all data were reported as the mean±standard error (SE) with three independent replications. Pearson correlations were drawn using SPSS software version 17.00 (SPSS Inc., Chicago, IL) among the different taste attributes and contributing biochemical contents to study the association among them.

Table S1

The average content of sodium, potassium, fluoride, and chloride in green tea infusions

Cultivar	Orthodox (mg L^{-1})				CTC (mg L^{-1})			
	Sodium	Potassium	Fluoride	Chloride	Sodium	Potassium	Fluoride	Chloride
TV1	1.09±0.03	149.60±3.92	0.67±0.03	27.00±1.53	1.39±0.13	239.45±2.79	0.88±0.02	33.00±1.53
TV9	0.95±0.06	177.64±6.94	0.78±0.02	11.23±0.89	1.32±0.12	281.63±5.93	0.96±0.05	13.58±0.36
TV18	2.08±0.07	164.05±3.65	0.50±0.02	15.83±1.09	2.25±0.15	242.00±2.17	0.62±0.03	23.17±0.95
TV20	1.66±0.07	158.41±2.56	0.58±0.02	18.07±0.79	2.18±0.10	237.55±4.53	0.77±0.02	22.30±0.44
TV22	1.07±0.04	175.62±3.96	0.87±0.03	9.90±0.78	1.23±0.03	263.63±7.12	1.12±0.04	11.80±0.91
TV23	1.73±0.11	179.53±3.05	0.76±0.05	12.80±0.61	2.63±0.11	220.03±4.23	1.08±0.04	20.43±0.92
TV25	0.97±0.07	168.12±3.98	0.83±0.02	12.13±0.59	1.52±0.06	236.05±4.13	1.07±0.06	15.77±0.38
HV39	1.31±0.12	89.25±2.74	0.43±0.03	12.73±0.18	1.35±0.08	138.67±4.72	0.59±0.03	16.67±0.35
RR17/144	1.09±0.08	165.80±4.63	0.61±0.01	21.63±0.95	1.21±0.02	217.33±4.49	0.85±0.02	31.23±1.01
Ging186	1.04±0.07	128.03±3.04	0.40±0.02	10.73±0.37	1.63±0.09	201.74±5.93	0.51±0.03	14.10±0.21
482/12	1.08±0.11	125.76±3.43	0.48±0.03	4.62±0.04	1.67±0.14	205.55±5.56	0.64±0.02	7.20±0.23

Values are 'mean±SE' of independent triplicate measurements.

References

1 C. D. Fernando and P. Soysa, *Springerplus*, 2016, **5**, 970.

2 ISO 14502-1:2005, *Determination of substances characteristic of green and black tea — Part 1 : Content of total polyphenols in tea — Colorimetric method using Folin-Ciocalteu reagent*, 2005.

3 ISO 14502-2:2005, *Determination of substances characteristic of green and black tea-Part 2. Contents of catechins in green tea; method using High Performance Liquid Chromatography. In International Standards Organizations Analytical Methods. ISO.*, 2005.

4 J. C. Too, T. Kinyanjui, J. K. Wanyoko and F. N. Wachira, *Food Nutr. Sci.*, 2015, **06**, 1014–1021.

5 ISO 9768:1994, *Determination of water extract*, 1994.

6 W. Samolińska, B. Kiczorowska, M. Kwiecień and E. Rusinek-Prystupa, *Biol. Trace Elem. Res.*, 2017, **175**, 495–502.

7 K. O. Moseti, T. Kinyanjui, J. K. Wanyoko and F. N. Wachira, *African Crop Sci. J.*, 2014, **22**, 897–904.

8 D. T. Waugh, W. Potter, H. Limeback and M. Godfrey, *Int. J. Environ. Res. Public Health*, 2016, **13**, 259.