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Chemical characteristics: tea flowers (Camellia sinensis) and black tea manufactured incorporating tea flowers

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Abstract

Tea is rich in biologically active compounds, and tea flowers have been reported to contain chemical constituents similar to those found in tea leaves. While the chemical characteristics of black tea and tea leaves from Sri Lankan tea cultivars have been extensively investigated, there is limited documentation on the chemical composition of tea flowers. Moreover, although tea flowers are occasionally incorporated into tea leaves during the production of specialty teas and black tea, the chemical properties of such blended teas have not been reported. This study aimed to evaluate the chemical composition of tea flowers, black tea manufactured with varying proportions of tea flowers, and tea flowers subjected to different drying methods. The results indicated that catechin and caffeine contents in black tea decreased with increasing incorporation of tea flowers, whereas polyphenol content remained relatively unchanged. Among the tested tea cultivars, tea flowers showed significant variation in catechin and caffeine contents, while polyphenol levels remained consistent. Additionally, oven-dried tea flowers retained significantly higher levels of catechins and caffeine compared to freeze-dried tea flowers, with no significant difference observed in polyphenol content. In conclusion, incorporating a lower percentage of oven-dried tea flowers into black tea is preferable to preserve catechin and caffeine content. These findings provide valuable insights for specialty tea manufacturers, emphasizing the importance of selecting appropriate drying methods and flower incorporation ratios to optimize the polyphenol and caffeine compositions of the made tea.

Keywords Chemical constituents · Drying of tea flowers · Tea cultivars · Tea flowers · Tea flowers incorporated black tea

Introduction

For centuries, Indigenous communities have employed plants and plant-derived substances for multiple purposes. Over time, the acceptance and utilization of plants have expanded significantly across various populations (Bargali et al. 2003; Padalia et al. 2015; Vibhuti et al. 2022). Currently, an estimated 80% of individuals in developing countries rely on traditional medicines and plant-based beverages for primary healthcare needs (Padalia et al. 2017; Parihaar et al. 2014). These plant-based remedies are frequently used

to treat both human and livestock ailments (Pathak et al. 2025). In recent decades, scientific interest in ethnobotany and the pharmacological potential of traditional plant uses has grown considerably on a global scale (Padalia et al. 2017; Pande et al. 2016). Concurrently, there has been a marked rise in the exploration and consumption of "nutraceuticals," also known as "functional foods," which are rich in phytochemicals believed to exert long-term therapeutic and health-promoting effects (Subramanian and Anandharamakrishnan 2023). Among these, tea has emerged as a widely recognized nutraceutical product due to its bioactive compounds and associated health benefits (Piyasena 2025).

Camellia sinensis, commonly known as the tea plant, is the source of one of the most widely consumed functional beverages globally. This beverage is primarily produced from the young, tender leaves of the tea plant (Piyasena et al. 2023a). Tea leaves are rich in a diverse range of bioactive compounds, including polyphenols, alkaloids, amino acids, carbohydrates, carotenoids, minerals, lignin, lipids, organic

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acids, and vitamins (Miao et al. 2023; Piyasena et al., 2023). Among these, polyphenols are particularly noted for their strong antioxidant activity, which underlies many of tea's therapeutic properties, such as anti-aging, anti-inflammatory, and anti-obesity effects (Tang et al. 2019). Regular tea consumption has been associated with a reduced risk of non-communicable diseases and is also believed to support immune function, digestive health, and oral hygiene (Piyasena et al. 2024; Piyasena 2025). While tea leaves are the primary component used in tea production, tea flowers have remained relatively underexplored despite their nutritional and pharmacological potential. In tea plantations where asexual propagation is practiced, tea flowers often compete with leaves for resources such as nutrients and water (Lin et al. 2003). Tea flowers are rich in bioactive compounds similar to those found in tea leaves, including polyphenols (such as catechins and flavonols), methylxanthines (e.g., caffeine), amino acids (notably theanine), proteins, polysaccharides, vitamins, and other phytochemicals, which collectively contribute to a wide range of health benefits (Chen et al. 2020a, b; Han et al., 2012; Wang et al. 2012; Yang et al. 2007; Yoshikawa et al. 2008). The majority of these biological activities are attributed to the presence of saponins, methylxanthine, polyphenols, and polysaccharides in tea flowers (Chen et al. 2018). Additionally, tea flowers contain a unique protease that significantly enhances the concentration of free amino acids in tea infusions, approximately doubling it compared to conventional methods. This enzyme exhibits superior catalytic efficiency compared to commercially available proteases (Chen et al. 2020a, b; Way et al. 2009). Despite their rich composition of secondary metabolites, tea flowers remain underutilized in the tea industry. Harnessing their bioactive compounds for the development of functional foods and value-added tea products could not only diversify product offerings but also reduce production costs and enhance resource utilization (Chen et al. 2018, 2020a, b; Joshi and Gulati 2011; Tang et al., 2022).

In countries such as India and China, tea flowers are often blended with tea leaves to produce specialty teas (Joshi and Gulati 2011). Similarly, in Sri Lanka, certain tea manufacturing factories incorporate tea flowers during the rolling stage of black tea production to enhance the quality of the final product. Despite these practices, the chemical composition of black tea enriched with tea flowers remains largely uncharacterized, as well as the chemical profiles of tea flowers from Sri Lankan tea cultivars, which have not yet been systematically studied. Therefore, the present study aimed to quantify key chemical constituents, total polyphenols, catechins, and caffeine, in black tea processed with varying proportions of incorporated tea flowers. In addition, the total polyphenol, catechin, sugar, and caffeine contents of tea flowers from different tea cultivars were evaluated.

Considering the influence of post-harvest treatment on phytochemical retention, the effects of different drying methods, specifically freeze-drying and oven-drying at 40 °C on the chemical composition of tea flowers were also assessed.

Materials and methods

Chemicals and reagents

Standards including caffeine, D-sucrose, gallic acid (GA), epigallocatechin (EGC), D-fructose, epicatechin (EC), epigallocatechin-3-gallate (EGCG), D-glucose, (+)-catechin (C), and epicatechin-3-gallate (ECG) were obtained from Sigma-Aldrich (Germany). Methanol (HPLC grade, 99.9%), sodium carbonate (anhydrous), Folin–Ciocalteu's phenol reagent, ethylenediaminetetraacetic acid (EDTA), ascorbic acid (purity 99.56%), and acetonitrile (HPLC grade, purity 99.99%) were also purchased from Sigma-Aldrich (Germany). An NH₂ column (150×4.6 mm, 5 μm) was used for chromatographic analysis. Ultrapure water was prepared using a Milli-Q system (Millipore, USA).

Sample collection and preparation

In February 2024, tea flowers from three distinct cultivars, TRI 2023, TRI 2043, and TRI 3031 were collected weekly over a three-week period from the tea germplasm maintained by the Tea Research Institute of Sri Lanka, located in Talawakelle (altitude≥1200 m; 6.9388° N, 80.6632° E), Sri Lanka. These tea cultivars were preserved as part of the Tea Research Institute's living germplasm collection. The respective accession numbers recorded for each tea cultivar were as follows: 2024/02/TRI 2023/01, 2024/02/TRI 2043/02, and 2024/02/TRI 3031/03. The tea plants were approximately 30 years old, and all flowers used in the study were harvested at 10 days post-anthesis. For sample preparation, 100 g of tea flowers from each tea cultivar were subjected to freeze-drying, while an additional 100 g were oven-dried at 40 °C for 24 h. Black tea samples were processed using a miniature black tea manufacturing unit (Teacraft, UK), incorporating tea flowers at varying proportions (5%, 10%, and 20%), alongside a control sample without flower incorporation. The processing parameters of the miniature manufacturing system were withering: wet and dry bulb temperature difference of 4 °C; relative humidity maintained at 75%; rolling: orthodox roller for 15 min with two maceration cycles; sieving: mesh size No. 8 (2.057 mm); fermentation: conducted in a controlled cupboard at dry bulb temperature of 25 °C, wet bulb temperature of 25 °C, and relative humidity of 100%; drying: fluidized bed dryer with inlet and outlet temperatures set at 125 °C and 95 °C,



respectively, for 18–21 min; fiber removal: separation using a fiber mat system.

Quantification of total polyphenolic contents

Approximately 0.200 ± 0.001 g of ground sample was accurately weighed and transferred into a 10 mL extraction tube containing 5 mL of 70% hot methanol. The mixture was vortexed using a vortex mixer (Lab-Line, USA) and incubated at 70 °C for 10 min. After cooling to room temperature, the samples were centrifuged at 3500 rpm for 10 min. The resulting supernatant was collected into graduated tubes. This extraction procedure was repeated, and the combined supernatants were transferred into a 10 mL volumetric flask and brought to volume with 70% methanol. For analysis, 1 mL of the extract was further diluted in a 100 mL volumetric flask with distilled water. From this diluted solution, 1 mL was transferred to a test tube and mixed with 5 mL of 10% Folin-Ciocalteu reagent. Within 3-8 min, 4 mL of 7.5% sodium carbonate solution was added. The mixture was incubated at room temperature for one hour. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). A calibration curve was prepared using gallic acid standards ranging from 10 to 60 mg/L. Results were expressed as a percentage by mass on a dry matter basis (w/w%). For determination of dry matter content, 5g of tea sample was accurately weighed into pre-weighed aluminum moisture cans and dried in a Memmert UND300 oven (Germany) at 103±2 °C for six hours until a constant weight was achieved. Dry matter percentage was calculated based on the weight difference before and after drying (ISO 1573:1980).

Quantification of individual catechins and caffeine

The extract prepared in Sect. "Quantification of total polyphenolic contents" was also used for the quantification of catechins and caffeine. A 1 mL aliquot of the extract was transferred into a graduated tube and diluted with 4 mL of a stabilizing solution containing 10% (v/v) acetonitrile, 500 μL/mL EDTA, and ascorbic acid. The mixture was homogenized and filtered through a 0.45 µm nylon membrane filter, and the filtrate was transferred to HPLC vials for analysis. High-performance liquid chromatography (HPLC) analysis was performed using an Agilent 1260 system (Agilent Technologies, Germany) equipped with a diode array detector (DAD). Separation was achieved on a Luna Phenyl-Hexyl column (250×4.6 mm, 5 μm) coupled with a Phenomenex Phenyl-Hexyl guard cartridge (4 mm × 3.0 mm). The column temperature was maintained at 35 °C. The injection volume was 10 µL, and the flow rate was set at 1.0 mL/ min. The mobile phase consisted of two solvents: mobile

phase A (2% v/v acetic acid, 20 μ g/mL EDTA, and 9% v/v acetonitrile) and mobile phase B (identical composition). The gradient elution program was as follows: 100% mobile phase A for the first 10 min, followed by a linear gradient to 68% A and 32% B over 15 min. This composition was held for 10 min before returning to 100% A for re-equilibration prior to the next injection. The total run time was 45 min. Catechins and caffeine were detected at 278 nm using a UV detector. Quantification was performed based on the comparison of retention times and peak areas with those of known standards (ISO14502-2:2005).

Extraction of sugars from tea flower

Freeze-dried or oven-dried tea flower samples (4 g) were extracted in 50 mL of distilled water at room temperature for 30 min using a magnetic shaker (LabTech, Korea), followed by filtration. A 1 mL aliquot of the resulting extract was then diluted with 4 mL of acetonitrile, filtered through a 0.45 µm nylon membrane filter (Agilent Technologies, Germany), and subjected to HPLC analysis. High-performance liquid chromatography (HPLC) was conducted using a 1260 HPLC system equipped with a Refractive Index Detector (RID) (Agilent Technologies, Germany). The injection volume was 10 µL, with a flow rate of 1.5 mL/min. The mobile phase consisted of 83% acetonitrile and 17% water, providing optimal resolution for sugar separation. An NH₂ column (Zorbax, Agilent Technologies) was employed and re-equilibrated with 100% acetonitrile prior to each run to ensure consistent column performance. The dry matter content of tea flower samples was determined by measuring weight loss at 103 °C. For calibration, sugar standards $(0.02\pm0.001 \text{ g})$ were accurately weighed and dissolved in 10 mL of deionized water to prepare standard solutions in the range of $100-1000 \mu g/mL$ (Nelum et al. 2023).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in Minitab (version 17). Mean separation was performed using Tukey's multiple comparison test at a significance level of $p \le 0.05$. All results are expressed as mean \pm standard deviation (SD), and differences were considered statistically significant at $p \le 0.05$, corresponding to a 95% confidence level.



Results and discussion

Total polyphenol, catechin, and caffeine contents of oven dried and freeze-dried tea flowers

The Tea Research Institute of Sri Lanka maintains over 600 tea accessions, and while the diversity of their metabolic profiles and selected biological activities has been extensively evaluated, the chemical characteristics of tea flowers remain largely unexplored (Basnayake et al. 2025; Kottawa-Arachchi et al. 2022; Piyasena et al. 2024, 2025). In this study, total polyphenol content (TPC), caffeine, and individual catechins, including EGCG, EGC, (+)-catechin, ECG, and EC, were quantified in freeze-dried and ovendried tea flowers. Tea flowers were obtained from three tea cultivars: TRI 2023, TRI 2043, and TRI 3031. Table 1 presents the TPC, catechin, and caffeine contents of both drying methods. Among the freeze-dried samples, there were no statistically significant differences in TPC among the three cultivars. However, the highest total catechin (TC) contents were observed in TRI 2023 (52.2 ± 0.9 mg/g) and TRI 3031 $(53.7\pm2.5 \text{ mg/g})$, with no significant difference between them. EGCG was the most abundant catechin across all cultivars, with concentrations in the order of TRI 3031>TRI 2023>TRI 2043. Gallic acid content was highest in TRI 3031 and lowest in TRI 2023. Conversely, caffeine content was highest in TRI 2023 (7.6 ± 0.1 mg/g) and lowest in TRI $3031 (5.5 \pm 0.1 \text{ mg/g})$. In oven-dried samples, no significant differences ($p \ge 0.05$) were observed in TPC or TC among the cultivars. EGCG remained the predominant catechin, with no significant variation among the cultivars. Additionally, gallic acid, caffeine, EC, ECG, and EGC levels did not differ significantly among the cultivars, except for catechin content.

In the TRI 2023 cultivar, there were no significant differences ($p \ge 0.05$) in TPC, TC, gallic acid (GA), and caffeine content between freeze-dried and oven-dried tea flower samples. Among the catechins analyzed, EGCG was the most

abundant, with no significant difference observed in EGCG content between the two drying methods. Notably, the highest gallic acid content in this tea cultivar was recorded in the oven-dried samples. For the TRI 2043 tea cultivar, no significant differences were observed in TPC and caffeine content between the freeze-dried and oven-dried samples. However, the freeze-dried tea flowers exhibited significantly higher TC compared to the oven-dried samples. EGCG remained the predominant catechin, and its content did not significantly differ between the two drying methods. Similarly, no significant differences were noted in the GA and caffeine contents between freeze-dried and oven-dried samples. In the TRI 3031 tea cultivar, TPC and caffeine content did not differ significantly between the two drying treatments. EGCG was again identified as the most abundant catechin. with the highest EGCG content (21.0 \pm 0.1 mg/g) observed in the TRI 3031 of freeze-dried samples. Overall, the results indicate that catechin and caffeine content in tea flowers were significantly influenced by both the tea cultivar type and the drying method employed. In TRI 2023, both freezedried and oven-dried tea flowers exhibited the highest TPC values, with no statistically significant differences between them.

The highest TPC was observed in the freeze-dried samples, which differed significantly ($p \le 0.05$) from the ovendried counterparts. Total catechin content also showed significant variation between treatments, with freeze-dried flowers exhibiting higher values than oven-dried flowers. Consistent with previous findings, epigallocatechin gallate (EGCG) was the predominant catechin, with the highest levels detected in freeze-dried samples, followed by oven-dried samples. In the TRI 3031 cultivar, the highest TPC was recorded in oven-dried tea flowers, followed by freeze-dried tea flowers. The greatest TC content ($2.10\pm0.01~\text{mg/g}$) was observed in TRI 3031 freeze-dried samples, whereas the lowest TC content was found in the oven-dried samples. Among the analyzed catechins, EGCG was the most abundant, with its highest concentration in the freeze-dried tea

Table 1 Total polyphenol, catechin, and caffeine contents (mg/g) of freeze dried and oven dried tea flowers

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Sample	GA	Caffeine	C	EC	ECG	EGC	EGCG	TC	TPC			
Freeze dried tea flowers												
TRI2023	$0.1 \pm 0.1^{c, B}$	$7.6\!\pm\!0.1^{a,A}$	$1.5 \pm 0.1^{a,A}$	$11.1\!\pm\!0.2^{a,A}$	$13.3 \pm 0.2^{b, A}$	$7.3\!\pm\!0.1^{a,A}$	$19.0 \pm 0.4^{b,A}$	$52.2 \pm 0.9^{a,A}$	$125.0\!\pm\!0.4^{a,A,B}$			
TRI2043	$0.2 \pm 0.1^{b, A'}$	$6.7 \pm 0.1^{b, A'}$	$1.1 \pm 0.1^{c, A'}$	$5.50 \pm 0.1^{c, A'}$	$12.5 \pm 0.1^{c, A'}$	$5.8 \pm 0.1^{a, A'}$	$16.9 \pm 0.1^{c, A'}$	$41.8 \pm 0.1^{b,A'}$	$118.9 \pm 1.5^{a, A'}$			
TRI3031	$0.3 \pm 0.1^{a,C^*}$	$5.5\!\pm\!0.1^{c,B*}$	$1.4\!\pm\!0.1^{b,A^*}$	$7.40\!\pm\!0.1^{b,A^*}$	$14.5\!\pm\!0.1^{a,A^*}$	$9.4\!\pm\!2.5^{a,A^*}$	$21.0\!\pm\!0.1^{a,A^*}$	$53.7\!\pm\!2.5^{a,A^*}$	$121.6\!\pm\!2.0^{a,A^*\!,B^*}$			
Oven dried tea flowers at 40°C												
TRI2023	$0.2\!\pm\!0.0^{a,A,B}$	$4.7\!\pm\!0.6^{a,A}$	$0.3 \pm 0.1^{a,A,B}$	$2.0\!\pm\!2.8^{a,B}$	$1.8\!\pm\!2.5^{a,B}$	$1.7\!\pm\!0.1^{a,A}$	$3.0\!\pm\!0.4^{a,B}$	$8.8 \pm 1.25^{a,B}$	$133.9 \pm 1.3^{a,A}$			
TRI2043	$0.5 \pm 0.1^{a, A'}$	$6.5 \pm 0.5^{a, A'}$	$0.4 \pm 0.1^{a, C'}$	$1.6 \pm 0.5^{a, C'}$	$2.3 \pm 0.1^{a, C}$	$0.9 \pm 0.1^{a, C'}$	$4.9 \pm 1.6^{a, C'}$	$10.1 \pm 2.1^{a, C'}$	$98.0 \pm 5.4^{b, B'}$			
TRI3031	$0.9\!\pm\!0.1^{a,B^*}$	$9.8 \pm 0.2^{a,A^*}$	$0.9 \pm 0.1^{a, B*}$	$5.5 \pm 0.2^{a,\mathrm{B*}}$	$6.4 \pm 0.7^{a, C*}$	$4.2\!\pm\!0.1^{a,A^*}$	$10.4 \pm 1.3^{a.B*}$	$27.4 \pm 2.2^{a, B*}$	$140.0 \pm 0.8.8^{a, A^*}$			

The values in the table are given as mean±SD of triplicates. The means that do not share the same subscription letter (within a column) are significantly different at 95% confidence (p≤0.05). Superscription lowercase letters denote the significant differences among different tea cultivars but within the same technique. Superscription uppercase letters denote the significant differences among different techniques but within the same cultivar (A, B, C-TRI 2023, A', B', C'-TRI 2043, A*, B*, C*-TRI 3031). Abbreviations: GA, Gallic Acid; C, Catechin; EC, Epicatechin; ECG, Epicatechin Gallate; EGC, Epigallocatechin; EGCG, Epigallocatechin Gallate; TC, Total Catechins; TPC, Total Polyphenol Contents



Table 2 Total polyphenol, catechin, and caffeine contents (mg/g) of black tea manufactured incorporating tea flowers

Sample	GA	Caffeine	С	EC	ECG	EGC	EGCG	TC	TPC
Black tea	2.1 ± 0.1^{b}	42.1 ± 0.5^a	0.4 ± 0.1^{a}	16.9 ± 0.2^a	6.7 ± 0.01^a	$2.7\!\pm\!0.01^{a}$	3.5 ± 0.1^{a}	30.2 ± 0.2^a	202.1 ± 0.9^{a}
5% TF in BT	$2.5 \!\pm\! 0.1^a$	39.9 ± 0.4^{b}	0.3 ± 0.1^{b}	$13.9\!\pm\!0.5^{b}$	$5.8\!\pm\!0.02^{b}$	$2.5\!\pm\!0.01^{b}$	$2.7\!\pm\!0.1^{b}$	$25.2\!\pm\!0.8^{b}$	$186.4\!\pm\!3.0^{a}$
10% TF in BT	1.7 ± 0.1^{c}	$38.7 \pm 0.1^{b, c}$	0.2 ± 0.1^{c}	11.3 ± 0.2^{c}	3.3 ± 0.01^{c}	2.0 ± 0.01^{c}	1.5 ± 0.1^{c}	18.2 ± 0.4^{c}	273.7 ± 12.0^a
20% TF in BT	$1,7 \pm 0.1^{c}$	38.1 ± 0.4^{c}	0.1 ± 0.1^d	10.0 ± 0.3^d	1.9 ± 0.01^d	1.7 ± 0.01^d	1.2 ± 0.1^{c}	14.9 ± 0.3^{d}	173.1 ± 2.3^a

The values in the table are given as mean \pm SD of triplicates. The means of different letters are significantly different at 95% confidence ($p \le 0.05$). Abbreviations: GA, Gallic Acid; C, Catechin; EC, Epicatechin; ECG, Epicatechin Gallate; EGC, Epigallocatechin; EGCG, Epigallocatechin Gallate; TC, Total Catechins; TPC, total polyphenol contents; TF, tea flowers; BT, black tea

flowers. These results indicate that polyphenol and caffeine levels in tea flowers vary significantly depending on both the cultivar and the drying method used. The TRI 2023 cultivar exhibited the highest TPC across both drying methods. Regarding caffeine content, the highest concentration was recorded in freeze-dried TRI 2023 flowers $(7.6\pm0.1\ mg/g)$. EGCG was the most prevalent catechin in all samples, consistent with profiles observed in tea leaves. Among the oven-dried samples, the highest caffeine content was found in TRI 3031 $(9.8\pm0.2\ mg/g)$.

According to previous reports, tea flowers contain chemical constituents comparable to those found in tea leaves, including approximately 7-11% tea saponins, less than 1% caffeine, 7–15% tea polyphenols, 1–3% flavonoids, 25-30% proteins, 20-35% total sugars, and 1-4% amino acids (Chen et al. 2018; Wang et al. 2010; Wu et al. 2023). In the present study, the caffeine content in both oven-dried and freeze-dried tea flower samples was found to be less than 1%, consistent with these previously reported values. The total polyphenol content ranged from 118.9 ± 1.5 to 125.0 ± 0.4 mg/g in freeze-dried samples and from 98.0 ± 5.4 to 140.0 ± 8.8 mg/g in oven-dried samples. TC content ranged from 41.8 ± 0.1 to 53.7 ± 2.5 mg/g in freeze-dried samples and from 8.8 ± 1.25 to 27.4 ± 2.2 mg/g in ovendried samples. As observed in tea leaves, EGCG was the most abundant catechin in tea flowers, with concentrations ranging from 16.9 ± 0.1 to 21.0 ± 0.1 mg/g in freeze-dried samples and from 3.0 ± 0.4 to 10.4 ± 1.3 mg/g in oven-dried samples (see Table 2). These findings are in good agreement with values reported in the existing literature.

In addition, it is stated that tea flowers contain a lower amount of catechins than tea leaves, and also catechin concentrations rise after budding, increasing as petals split, and then reducing to a minimum after the full bloom of the tea flower. Furthermore, different organs of a tea flower consist of distinct components. The calyx contains more EGCG than other tea flower parts (Chen et al. 2018; Joshi and Gulati 2011). The aglycones of the major flavonols present in tea leaves include myricetin, quercetin, and kaempferol, which are also found in tea flowers. Apart from major flavonols, chakaflavonoside was reported in tea flowers. And also, 12 flavonols have been identified from tea flowers (Chen et al. 2018). Gas chromatography-mass spectrometry

analysis revealed that tea flowers were comprised of 21 volatile components and nine fatty acids (Sharma et al., 2022). Moreover, theanine is the most prevalent free amino acid in tea flowers, and the amount of free amino acids in the flowers comprises 0.8% of the dry weight of the flowers (Chen et al. 2018). There have been 25 saponins identified in tea flowers. Tea flowers have higher concentrations of saponins than tea leaves, containing 0.47-4.23% of dry weight, and these saponins exhibited remarkable biological activities (Matsuda et al. 2016; Morikawa et al. 2012). Additionally, it has been reported that proteins constitute approximately 30–50% of the dry weight of tea flowers (Chen et al. 2018). Among the various catechins present, EGC, ECG, and EGCG are identified as the predominant catechins in tea flowers across different cultivars and geographical regions, although the specific concentrations and profiles of catechin derivatives may vary depending on the origin (Chen et al. 2018; Morikawa et al. 2013). The caffeine present in tea flowers is synthesized in situ, rather than being translocated from the tea leaves (Fujimori and Ashihara 1993). The antioxidant capacity of tea flowers has been linked to both polysaccharides and catechins (Chen et al. 2018; Tang et al., 2019). Furthermore, due to their substantial catechin content and relatively low levels of caffeine, tea flowers have been proposed as a potential ingredient for the development of novel tea-based beverages (Chen et al. 2018).

Total polyphenol, catechin, and caffeine contents of black tea manufactured incorporating tea flowers

In some tea factories, tea flowers are traditionally added during the rolling stage of black tea processing to enhance tea quality. Additionally, tea organizations in China and India have reportedly used processed fresh tea flowers to impart flavor to specialty teas (Joshi and Gulati 2011). However, limited research has been conducted on the chemical composition of black tea produced with the incorporation of tea flowers. In this study, tea flowers were incorporated at different ratios (5%, 10%, and 20%) during the rolling stage of black tea manufacture, and the resulting teas were analyzed for TPC, catechins, and caffeine. The results are summarized in Table 2. Incorporation of tea flowers led to significantly lower levels of catechins and caffeine compared to



conventional black tea. Notably, only the gallic acid content in the 5% flower-incorporated black tea was significantly higher than in the conventional black tea. The 5% tea flower-incorporated tea also exhibited significantly higher concentrations of (+)-catechin, EC, ECG, EGC, EGCG, and TC than the 10% and 20% incorporation levels. Significant differences in gallic acid and caffeine content were observed between the 10% and 20% flower-incorporated teas. Furthermore, all catechins except EGCG were significantly reduced in the tea containing 20% tea flower compared to black tea as well as teas with 5% and 10% tea flower incorporation. No significant differences in total polyphenol content were found among the samples. In conclusion, increasing the proportion of tea flower incorporation during black tea manufacture results in a decrease in catechin and caffeine content, while total polyphenol levels remain unaffected.

Glucose, sucrose and fructose contents of tea flowers

In the present study, the concentrations of sucrose, glucose, and fructose in freeze-dried tea flowers were quantified using high-performance liquid chromatography (HPLC). The results indicated that the fructose, glucose, and sucrose contents of tea flowers were 85.89 ± 1.31 mg/g, 95.29 ± 5.72 mg/g, and 84.66 ± 1.78 mg/g, respectively. In comparison, the average sugar concentrations reported for Sri Lankan black tea were substantially lower: sucrose at 9.53 ± 3.46 mg/g, glucose at 8.79 ± 2.58 mg/g, and fructose at 8.17 ± 1.98 mg/g (Piyasena et al. 2022, 2023b). Moreover, sugar concentrations were found to vary significantly across different stages of black tea processing. The highest sucrose content (32.03±3.29 mg/g) was observed during the withering stage, while the highest glucose $(9.63\pm3.46 \text{ mg/g})$ and fructose (9.22±1.43 mg/g) levels were observed in fully processed black tea (Piyasena et al. 2023b). Additionally, freeze-dried harvestable tea shoots (buds and the first two leaves) were reported to contain considerably lower sugar levels: sucrose at 0.97 ± 0.77 mg/g, glucose at 5.99 ± 2.98 mg/g, and fructose at 6.77 ± 1.68 mg/g (Piyasena et al., 2023). These findings clearly indicate that tea flowers contain significantly higher concentrations of sucrose, glucose, and fructose compared to tea leaves and black tea at various processing stages. This observation is consistent with previous reports suggesting that tea flowers possess a substantially greater total sugar content than tea leaves (Wei et al. 2010). It has been reported that saccharides constitute approximately 20–30% of the dry weight of tea flowers (Chen et al. 2018).

Despite the comprehensive nature of this study, several limitations should be acknowledged and addressed in future

research. The relatively small sample size may limit the generalizability of the findings. To enhance the robustness of future studies, it is recommended to incorporate larger sample sizes and prepare a broader range of black tea samples with varying proportions of tea flower incorporation, followed by detailed biochemical analyses. Although over 70 cultivars are recommended for commercial tea cultivation in Sri Lanka, this study utilized flowers from only three cultivars. Therefore, future research should include a wider selection of cultivars to capture the full spectrum of biochemical diversity. Comprehensive profiling of key biochemical constituents, including caffeine, catechins, polyphenols, amino acids, saponins, and tea polysaccharides, as well as assessments of biological activities, is warranted. Additionally, the observed biochemical variability in tea flowers may have been influenced by uncontrolled environmental and seasonal factors, which should be taken into consideration in subsequent investigations.

Conclusion

Tea flowers, traditionally considered an agricultural byproduct, have recently been recognized as a potential functional food source. They are rich in bioactive compounds such as saponins, polyphenols, polysaccharides, and functional proteins, which contribute to their health-promoting properties. As a result, tea flowers have been utilized in the development of various functional food products. In addition to their use in food, some tea producers incorporate tea flowers into specialty tea formulations. Notably, tea flowers are sometimes added during black tea processing to enhance product quality. In the present study, the incorporation of tea flowers into black tea resulted in significantly lower catechin and caffeine contents compared to black tea, while total polyphenol content remained unchanged. Variations in catechin and caffeine concentrations were observed among tea flower samples from different cultivars, whereas polyphenol content showed no significant differences. Moreover, oven-dried tea flowers exhibited significantly higher levels of catechins and caffeine compared to freeze-dried samples. These findings offer valuable insights for specialty tea producers, highlighting the importance of selecting appropriate drying methods and optimizing the proportion of tea flower incorporation to achieve desired biochemical and sensory profiles.

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Declarations

Ethical statement This is to inform you that in this study, we have not been involved in any animal and human studies.

Plant ethics: compliance with ethical standards The collection of the leaves of *Camellia sinensis* L. O. (Kuntze) used in this study complied with local or national guidelines. Leaves of *Camellia sinensis* L. O. (Kuntze) were collected in the National Tea Germplasm, Tea Research Institute of Sri Lanka. As tea is a commercially grown plant, a special license or special permission is not required to collect the tea leaves.

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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