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Article

Nutritional composition, polyphenolic content and biofunctional potential of household spent tea leaves for sustainable food utilization

Said Dahmouni[1], Zineb Bengharbi[1], Djilali Benabdelmoumene[1*], Salim Nebbache[1], Wasim S. M. Qadi[2], Ahmed Mediani[2]

[1] Applied animal physiology lab, Abdelhamid Ibn Badis university, Mostaganem 27000, Algeria.
[2] Institute of Systems Biology (INBIOSIS), Universiti Kebangsaan Malaysia (UKM), Bangi 43600, Malaysia.
*Correspondence: Djilali Benabdelmoumene; Djilali.benabdelmoumene@univ-mosta.dz

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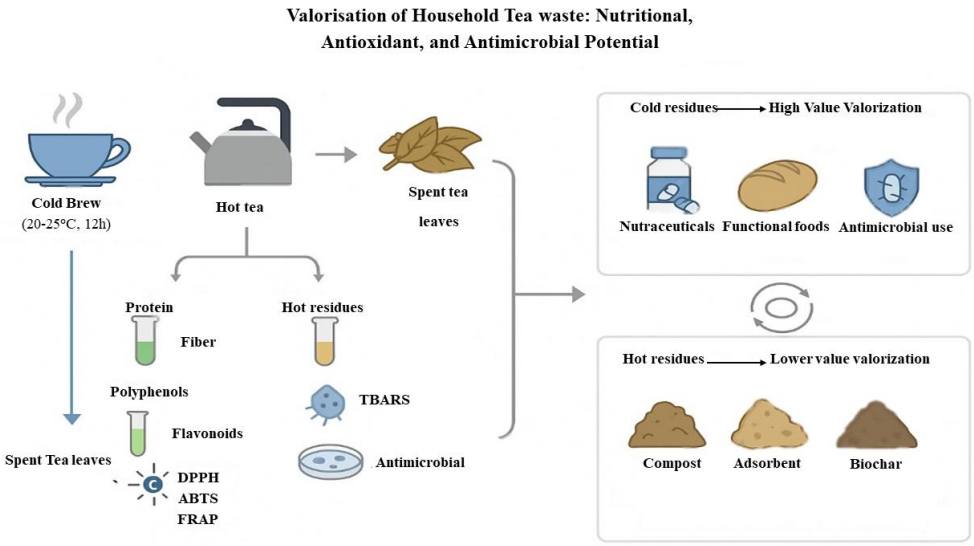
ABSTRACT

Tea ranks among the most widely consumed beverages globally, yet its preparation yields substantial quantities of spent leaves each year, which are typically discarded despite still containing significant levels of bioactive constituents. Here, we investigate the nutritional composition, antioxidant capacity, and antimicrobial potential of household tea residues under contrasting infusion conditions. Commercial green teas were brewed at low (2025 °C, 12 h) and high (60–100 °C, 3–5 min) temperatures to simulate domestic practices. Cold-brew residues preserved higher levels of protein (≈20.5–21.0 g/100 g DW), dietary fiber (≈45–46% DW), and polyphenols (≈106–109 mg GAE/g DW), along with stronger antioxidant retention (>90% of baseline DPPH, ABTS, FRAP) and broader antimicrobial activity, particularly against *Staphylococcus aureus*. In contrast, hot-brew residues showed progressive depletion of phenolics (down to 85 mg GAE/g DW) and diminished radical-scavenging and antibacterial capacity, while lipid peroxidation markers (TBARS) nearly doubled at 100 °C. These findings reveal a dual role of infusion temperature: enriching the beverage but depleting the residue, with consequences for valorization potential. From a circular bioeconomy perspective, cold-brew residues represent superior substrates for high-value recovery of antioxidants, fiber, and antimicrobials, whereas hot-brew residues may require advanced extraction or redirection to lower-value applications. These findings position spent tea leaves as a valuable resource for developing nutrient-dense foods, health-oriented bioactive ingredients, and low-impact, sustainable bioproducts.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Tea is among the most widely consumed beverages globally, and its cultivation and preparation generate considerable quantities of solid residues (Çakmak et al., 2024; Seth et al., 2025). Black and green tea together represent nearly 70–80% of total tea production (Tanaka & Matsuo, 2020). Recent estimates indicate that China alone discards more than 5 million tonnes of used tea leaves annually, underscoring the magnitude of this underutilized biomass (Unyay et al., 2025; Sagar et al., 2025). Traditionally, these residues have been considered waste; however, recent studies highlight that spent tea leaves retain significant levels of proteins, dietary fiber, caffeine, and polyphenols (Harfoush et al., 2024; Mandal et al., 2024). Recovering and valorizing these nutrients not only reduces environmental pollution but also contributes to the circular economy by transforming a major waste stream into a resource with nutritional and industrial value (Bekavac et al., 2025;Jayakala Devi et al., 2024).

Nutritional characterization of spent tea leaves reveals valuable proximate components including moisture content, ash, crude fat, and total dietary fiber. These parameters are crucial for assessing their potential as food additives or supplements, offering nutritional value while reducing waste. Spent tea leaves are also particularly rich in bioactive compounds such as total phenolic content (TPC), total flavonoid content (TFC), and condensed tannins. The biochemical profile depends strongly on brewing conditions, including infusion temperature, steeping duration, particle size, and water composition. High-temperature and prolonged brewing enhance the release of polyphenols and caffeine into the infusion, decreasing residual concentrations, while cold brews often leave higher proportions of certain polyphenols and minerals in the leaves (Carloni, Girolametti, et al., 2023; Winiarska-Mieczan & Baranowska-Wójcik, 2024). Such compounds underpin the potential of tea residues in functional foods, nutraceuticals, and cosmetics (Zahra et al., 2022; Ozsefil & Ziylan-Yavas, 2023).

The antioxidant activity of tea residues is commonly evaluated using DPPH radical scavenging and ABTS assays. These tests confirm that tea residues retain substantial antioxidant potential, influenced by brewing method and solvent extraction. Incorporating spent tea leaf powder into baked goods, for example, enhances antioxidant activity without compromising sensory quality (Koh et al., 2023). Beyond antioxidant properties, tea residues also exhibit notable antimicrobial activity. Extracts from green and black tea residues, optimized for polyphenol recovery, inhibit Gram-positive bacteria such as S. aureus and Bacillus subtilis, though activity against Gram-

negative strains is weaker (Alghamdi, 2023; Harfoush et al., 2024). Efficacy varies with solvents, concentration, and residual phenolic content, with catechins and flavonoids playing central roles (Çakmak et al., 2024).

A circular economy treats waste not as an end point but as an input to new value chains, keeping materials "in circulation" rather than discarding them. Agricultural residues such as spent tea leaves become feedstocks for new products, moving away from the linear "take-make-waste" paradigm. Recent studies highlight multiple valorization routes: as catalysts in wastewater treatment, as biochar for pollution adsorption and soil improvement, as biofertilizers or animal feed, and as bioenergy sources (Moreno-Bermedo et al., 2025; Hao et al., 2025; Powrel et al., 2025). Such pathways strongly support sustainability and circular bioeconomy goals. Although, consumers are increasingly aware of health risks from contaminants in tea. Infusion extracts mainly soluble compounds, whereas the decoction, common in India, China, and Egypt, also releases insoluble constituents, potentially raising contaminant levels. Accordingly, emerging strategies combine advanced monitoring of contaminants with green extraction technologies and residue valorization, ensuring both consumer safety and alignment with sustainable food system goals.

This study puts forward the notion that brewing temperature exerts a decisive influence on the retention of nutrients and bioactive constituents in spent tea leaves, thereby shaping their antioxidant and antimicrobial capacities and governing their suitability for value-added applications. Accordingly, this work investigates how infusion parameters (namely temperature and steeping duration) modulate the residual biochemical composition and functional properties of spent leaves. Emphasis was placed on characterising shifts in physicochemical traits, phenolic compound profiles, antioxidant activity, and antimicrobial responses to generate mechanistic evidence supporting the valorisation of this underutilised biomass within food and bioprocessing frameworks.

2. METHODOLOGY

2.1 Infusion parameters and sample preparation

Commercial green teas were obtained from local retailers to represent household consumption. Uninfused leaves served as controls, reflecting native composition prior to extraction (Çakmak et al., 2024). Household-style infusions were prepared under two conditions to simulate domestic practices, given the influence of temperature and time on phenolic and antioxidant retention (Vinci et al., 2022; Oracz et al., 2025): cold infusions at 20°C, 22°C and 25 °C for 12 h under refrigerated or ambient conditions, and hot infusions at 60, 70, and 100 °C for 3–5 min with potable water. Spent leaves were recovered by sterile filtration, cooled to room temperature, oven-dried at 40 °C to constant weight, milled (<500 µm), and homogenized.

2.2 Tea leaves Residues Analysis

Moisture content was determined by oven-drying 2 g of finely ground tea powder at 105 °C to constant weight, with results expressed as a percentage of the initial sample weight. Reported values for dried residues typically range between 10 and 15% (Dahmouni et al., 2025). Ash content was measured by incinerating 2 g of dried powder at 550 °C in a muffle furnace until a whitish residue was obtained, representing the inorganic mineral fraction, which generally accounts for 4–6% of tea residues (Ateş et al., 2023; Wu et al., 2024; Duranay et al., 2025). Crude protein was quantified using the Kjeldahl method, involving digestion, neutralization, distillation, and titration, with nitrogen values converted using the factor N × 6.25; protein levels usually range from 15 to 25%, supporting potential applications in feed and food fortification (Kumar et al., 2023). Crude fat was determined by Soxhlet extraction with petroleum ether (40–60 °C) for six hours, and expressed as a percentage of dry matter. Tea residues typically contain 4–6% fat, reflecting their retention of lipophilic compounds such as pigments and essential oils that influence sensory and functional properties (Aydemir et al., 2024). Total dietary

fiber (TDF), including soluble (SDF) and insoluble (IDF) fractions, was assessed by the enzymatic–gravimetric method involving sequential digestion with α-amylase, protease, and amyloglucosidase, followed by filtration and ethanol precipitation. Both fractions were corrected for protein and ash, and results expressed on a dry weight basis. Tea residues may contain up to 40–50% TDF, dominated by insoluble fiber, highlighting their potential as a dietary fiber source for food applications (Nguyen et al., 2022; Dhar et al., 2025).

Dried tea residues obtained from each infusion treatment were extracted prior to TPC, TFC, tannin, antioxidant, and antimicrobial analyses. Briefly, 1 g of finely ground residue was mixed with 20 mL of 80% methanol (v/v) and subjected to maceration for 24 h at room temperature under continuous agitation (150 rpm). The mixture was then centrifuged at 5000 rpm for 10 min, and the supernatant was collected (Sanaka et al., 2005). The pellet was re-extracted under identical conditions, and both supernatants were combined and filtered through a 0.45 µm membrane filter. Extracts were stored at 4 °C in amber vials and used for TPC, TFC, tannin content, antioxidant assays (DPPH, ABTS, FRAP, TBARS), and antimicrobial testing.

2.3 Phenolic, Flavonoid, and Tannin Contents

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric assay, where tea extracts were reacted with Folin–Ciocalteu reagent and sodium carbonate, incubated in the dark, and measured at 765 nm; results were expressed as mg gallic acid equivalents (GAE) per g dry weight (Bouhalla et al., 2024). Total flavonoid content (TFC) was quantified by the aluminium chloride method, involving sequential addition of $NaNO_2$, $AlCl_3$, and NaOH, with absorbance measured at 510 nm and results expressed as mg quercetin equivalents (QE) per g dry weight (Nguyen et al., 2022). Condensed tannins (proanthocyanidins) were assessed by the vanillin–HCl method, where extracts were mixed with vanillin and HCl, incubated in the dark, and measured at 500 nm; catechin served as the calibration standard, and results were expressed as mg catechin equivalents (CE) per g dry weight (Ahmed et al., 2023).

2.4 Tea Residues Antioxidant Activity

Antioxidant activity of tea residue extracts was evaluated through complementary assays (Solomon et al., 2025). For the DPPH radical-scavenging assay, extracts were reacted with 0.1 mM DPPH solution, incubated for 30 min in the dark, and measured at 517 nm, with results expressed as percentage inhibition (%) and validated using Trolox, quercetin, and gallic acid as positive controls (Bouhalla et al., 2024). The ABTS assay was performed by mixing extracts with the $ABTS^{+}\bullet$ working solution and recording absorbance at 734 nm after 6 min, expressing antioxidant activity as Trolox equivalents (Ahmed et al., 2023). The FRAP assay was carried out using the TPTZ–$FeCl_3$ reagent with absorbance measured at 593 nm and results expressed as µmol Trolox equivalents per g dry weight. Lipid peroxidation was assessed by the TBARS method, where extracts were incubated with TCA–TBA–HCl reagent, heated, centrifuged, and measured at 532 nm, with values expressed as nmol MDA per g dry weight (Botsoglou et al., 1994). Positive controls were included across assays to ensure validation and comparability of antioxidant responses.

2.5 Antimicrobial Activity

Pathogenic strains including Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 33862, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 10231, and Klebsiella pneumoniae ATCC E47 IV.2.2 were reactivated in Brain Heart Infusion Broth (BHIB) and incubated at 37 °C for 24 h to confirm purity and viability. For antagonism assays, cultures were transferred into fresh BHIB and incubated under the same conditions to obtain actively growing cells (Al-Qahtani et al., 2024; Çakmak et al., 2024; Jadhav et al., 2025). Tea residue extracts were prepared as described previously and concentrated to dryness, then re-dissolved in sterile 10%

DMSO to obtain a stock solution of 100 mg/mL. Working solutions of 25, 50, and 100 mg/mL were prepared by serial dilution. Microbial suspensions were standardized by adjusting OD_{600} to 0.08–0.10 (≈1 × 10^8 CFU/mL) (Qadi et al., 2023). For each test, 100 µL of standardized inoculum was spread onto Mueller–Hinton agar plates, and 10 µL of each extract concentration was applied into wells (6 mm diameter). Plates were incubated at 37 °C for 24 h, and antimicrobial activity was expressed as the diameter of inhibition zones (mm).

2.6 Statistical Analysis

All experiments were conducted in triplicate (n = 3), and results are reported as mean ± standard deviation (SD). Data were first tested for normality and homogeneity of variances. For normally distributed data with equal variances, one-way analysis of variance (ANOVA) was performed, followed by Tukey's HSD post hoc test to assess pairwise differences. When these assumptions were not satisfied, the nonparametric Kruskal–Wallis test with Dunn's post hoc correction was applied. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

3 RESULTS AND DISCUSSION

3.1 Chemical Composition of Tea Residues

Table 1 presents composition of spent green tea leaves was significantly influenced by infusion temperature and duration, confirming that domestic brewing conditions modulate not only the chemical profile of the beverage but also the valorization potential of the residues. Significant differences ($p < 0.05$) among treatments are indicated by superscript letters.

Table 1 Proximate composition of tea residues (moisture, ash, crude protein, crude fat, and dietary fiber fractions) under different extraction temperature conditions.

Parameter	Control	Cold 20 °C	Cold 22 °C	Cold 25 °C	Hot 60 °C	Hot 70 °C	Hot 100 °C
Moisture (g/100 g FW)	8.87 ± 0.21^a	9.24 ± 0.28^a	9.36 ± 0.25^a	9.58 ± 0.22^a	10.12 ± 0.27^b	10.46 ± 0.24^b	11.03 ± 0.31^c
Ash (% DW)	5.38 ± 0.18^a	5.29 ± 0.12^a	5.21 ± 0.14^a	5.12 ± 0.11^a	4.88 ± 0.17^b	4.77 ± 0.15^b	4.61 ± 0.19^c
Crude protein (g/100 g DW)	21.76 ± 0.58^a	21.18 ± 0.52^a	20.92 ± 0.47^b	20.54 ± 0.49^b	19.73 ± 0.41^c	19.26 ± 0.53^c	18.47 ± 0.62^d
Crude fat (g/100 g DW)	4.23 ± 0.19^a	4.12 ± 0.17^a	4.05 ± 0.16^a	3.91 ± 0.18^a	3.64 ± 0.20^b	3.37 ± 0.15^b	3.09 ± 0.21^c
Total dietary fiber (% DW)	46.47 ± 1.02^a	46.03 ± 0.93^a	45.72 ± 0.88^a	45.18 ± 0.95^b	44.02 ± 0.91^c	43.48 ± 0.84^c	42.26 ± 1.07^d
Insoluble DF (% DW)	37.82 ± 0.87^a	37.39 ± 0.82^a	37.15 ± 0.76^a	36.72 ± 0.85^b	35.48 ± 0.79^c	35.03 ± 0.73^c	34.07 ± 0.88^d
Soluble DF (% DW)	8.65 ± 0.28^a	8.64 ± 0.24^a	8.57 ± 0.23^a	8.42 ± 0.27^a	8.14 ± 0.22^b	7.91 ± 0.21^b	7.54 ± 0.26^c

Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences at $p < 0.05$ according to Tukey's HSD test

Moisture content increased steadily from the uninfused leaves (~8.9 g/100 g FW) to the hot-infused residues (>11 g/100 g FW at 100 °C). This trend can be explained by the thermal disruption of cell walls, solubilization of structural polysaccharides, and denaturation of proteins, which together enhance porosity and create hydrophilic sites that retain water. Comparable observations have been reported during black tea processing,

where moisture levels decreased from 76.4% to 63.8% during withering, reflecting the strong influence of structural integrity on water retention and release (Jabeen et al., 2019). For dried tea residues, equilibrium values of ~6.8–7.5% have been reported for tea dust and leaf fragments, highlighting the typical stability threshold of these byproducts (Shchegoleva et al., 2021). Importantly, residual moisture above 10% is generally considered critical, since it increases microbial susceptibility and requires stabilization by oven- or freeze-drying to ensure safe storage. At the same time, the high water-holding capacity of spent tea leaves may be advantageous in functional food applications. For example, incorporating 8% spent tea leaf powder into gluten-free shortbread cookies increased final moisture from 1.62% in controls to ~2.18–2.35%, an effect attributed to the water-binding capacity of tea fibre (Koh et al., 2023). Thus, while elevated moisture in hot-infused residues presents a challenge for shelf life and processing costs, it also represents a functional opportunity for valorization in food and agricultural systems, provided drying and stabilization are adequately managed.

Ash levels decreased progressively with infusion severity, from 5.4% DW in the uninfused leaves to ~4.6% DW after brewing at 100 °C. This decline reflects the leaching of soluble minerals (notably potassium, calcium, and magnesium) into the infusion liquor. Comparable ash fractions of ~5.8–6.2% have been reported for dried tea dust and leaf tea (Shchegoleva et al., 2021), confirming that our baseline values fall within expected ranges. Variations of up to 1–1.5% have been documented depending on processing stages, with withering and fermentation promoting solubilization of cations (Jabeen et al., 2019). From a valorization perspective, reduced ash content lowers the mineral density of residues destined for feed or nutraceutical use, but it may still support applications as soil amendments or compost fortifiers, given their persistent inorganic fraction.

Crude protein content exhibited a marked dependence on infusion temperature. Uninfused control leaves contained ~21.8 g/100 g DW, reflecting the native nitrogenous profile of green tea. Residues from cold infusions (20–25 °C, 12 h) retained between ~20.5 and 21.0 g/100 g DW, indicating only minor nitrogen losses under low-temperature conditions. In contrast, hot infusions (60–100 °C, 3–5 min) resulted in significantly greater depletion, with protein levels declining to ~19.7 g/100 g DW at 60 °C, ~19.2 g/100 g DW at 70 °C, and ~18.5 g/100 g DW at 100 °C. This gradient reflects the temperature-driven migration of soluble nitrogenous compounds into the infusion liquor. At lower temperatures, solubilization of free amino acids and small peptides remains limited, thereby preserving the bulk of proteinaceous material within the biomass. By contrast, elevated temperatures promote protein denaturation, hydrolysis, and subsequent solubility, enhancing nitrogen extraction into the beverage and depleting the residue. A similar pattern has been observed in black tea processing, where the concentration of free amino acids decreases significantly during withering and fermentation under thermal stress, particularly for glutamic acid and serine (Jabeen et al., 2019). Our values are consistent with proximate analyses of spent tea powders repurposed in bakery applications, where crude protein was reported at ~17–19 g/100 g DW depending on processing severity (Koh et al., 2023). From a valorization perspective, cold-brew residues represent a more protein-dense byproduct, enhancing their suitability for incorporation into protein-enriched food formulations, functional feed supplements, or nutraceutical products. Conversely, hot-brew residues, though poorer in protein, remain viable for lower-value applications where nitrogen contribution is less critical, such as soil amendments, compost, or adsorbent matrices.

Dietary fiber was the most abundant fraction in spent green tea leaves, ranging from ~46.5% DW in uninfused controls to ~42.3% DW in residues from hot infusions. Cold-brew treatments (20–25 °C, 12 h) retained higher levels (~45.7–46.0% DW), whereas hot infusions progressively reduced fiber content (44.0% at 60 °C, 43.5% at 70 °C, and 42.3% at 100 °C). This reduction reflects the temperature-dependent solubilization of hemicelluloses and pectins, which partially migrate into the infusion liquor, while more recalcitrant fractions such as cellulose and lignin remain bound in the biomass (Shchegoleva et al., 2021; Jabeen et al., 2019). Insoluble dietary fiber

(IDF) dominated the composition (35–38% DW), while soluble dietary fiber (SDF) contributed 8–9% DW, a ratio consistent with previous findings on tea residues and other polyphenol-rich byproducts (Elleuch et al., 2011; Koh et al., 2023). Similar TDF values have been reported in fiber-rich agro-industrial residues such as fruit pomace and cereal brans, ranging from 40 to 60% DW, confirming the high-fiber potential of tea residues for food applications (Gupta et al., 2011; Ajila & Rao, 2013). From a nutritional perspective, the predominance of IDF supports gastrointestinal motility and fecal bulking, while SDF provides fermentable substrates for gut microbiota, yielding short-chain fatty acids that modulate host metabolism (Anderson et al., 2009; Fuller et al., 2016; Slavin, 2013). These functions have been validated in studies where fiber-rich food byproducts improved gut microbiota diversity and reduced glycemic response (Gupta et al., 2011; Elleuch et al., 2011). From a technological standpoint, the water-holding capacity and swelling properties of tea fibers improve dough rheology and texture in bakery products, as shown by (Koh et al., 2023), who reported that incorporation of 8% spent tea leaf powder into gluten-free cookies increased dietary fiber content and final product moisture retention. Comparable results have been achieved with apple pomace and citrus peel fibers, demonstrating the broader applicability of tea residues as functional food ingredients (Ajila & Rao, 2013; Larrauri, 1999).

From a technological and valorization perspective, these temperature-dependent shifts in protein and fiber content help position cold residues as richer substrates for functional applications, while hot residues remain suitable for lower-value uses. It is important to note that the six selected infusion temperatures (20, 22, 25 °C for cold brewing and 60, 70, 100 °C for hot brewing) were intentionally chosen to represent realistic domestic practices and to capture distinct extraction regimes described in recent studies (Carloni, Albacete et al., 2023; Oracz et al., 2025). Within each infusion method, the temperature increments were designed to reveal intra-group differences in extraction behavior: in cold brews, the mild increase from 20 to 25 °C resulted in subtle but significant decreases in protein, minerals, and fiber due to slightly enhanced solubilization, whereas in hot brews, the stronger gradient from 60 to 100 °C progressively intensified extraction and thermal degradation phenomena. These intra-group variations directly reinforce our research objective by showing that not only the brewing mode (cold vs. hot) but also the specific temperature selected within each mode critically determines the nutritional and functional profile of the resulting residues.

3.2 Total phenolic content

The TPC of spent green tea leaves exhibited a clear dependence on brewing temperature (**Table 2**). Uninfused leaves contained the highest TPC (112.34 mg GAE/g DW), reflecting the native polyphenolic reservoir of green tea. Residues from cold infusions (20–25 °C, 12 h) retained a large fraction of these compounds, with only modest decreases (105.83–108.92 mg GAE/g DW), suggesting that low-temperature steeping has limited extraction efficiency and preserves a significant proportion of phenolics in the biomass. By contrast, hot infusions (60–100 °C, 3–5 min) promoted progressive depletion of phenolics from the leaves into the liquor, with TPC values declining from 96.41 mg GAE/g DW at 60 °C to 85.36 mg GAE/g DW at 100 °C.

Table 2 Total phenolic content (TPC), total flavonoid content (TFC), and condensed tannins of spent green tea leave under different infusion conditions

Parameter	Control	Cold 20 °C	Cold 22 °C	Cold 25 °C	Hot 60 °C	Hot 70 °C	Hot 100 °C
TPC (mg GAE/g DW)	112.34 ± 2.85^{a}	108.92 ± 2.41^{a}	107.65 ± 2.73^{a}	105.83 ± 2.26^{a}	96.41 ± 2.55^{b}	91.78 ± 2.38^{c}	85.36 ± 2.14^{d}
TFC (mg QE/g DW)	38.26 ± 1.12^{a}	37.45 ± 1.07^{a}	36.82 ± 1.14^{a}	36.11 ± 1.09^{a}	31.27 ± 0.95^{b}	28.93 ± 0.91^{c}	25.68 ± 0.88^{d}

Condensed tannins (mg CE/g DW)	22.14 ± 0.78[a]	21.65 ± 0.72[a]	21.31 ± 0.75[a]	20.87 ± 0.69[a]	17.93 ± 0.63[b]	16.82 ± 0.59[c]	14.74 ± 0.55[d]

Values are mean ± SD (n = 3). Different superscript letters within a row indicate significant differences ($p < 0.05$).

These patterns can be attributed to the temperature-driven solubilization and diffusion of polyphenols, combined with partial thermal degradation of labile compounds at high brewing temperatures. Similar observations have been reported for tea infusions, where hot brewing significantly increases phenolic release into the beverage relative to cold brewing (Damiani et al., 2014). This aligns with kinetic models showing that low-temperature extractions proceed more slowly, thereby leaving higher residual phenolic levels in the spent matrix (Oracz et al., 2025). Importantly, the persistence of substantial phenolic fractions in residues even after brewing supports their valorization as sources of antioxidant compounds (Çakmak et al., 2024). The magnitude of residual TPC in our hot-brew residues is comparable with recent reports of tea byproducts, where values of 80–100 mg GAE/g DW have been measured under aqueous infusion, and markedly lower than those obtained using optimized solvent extractions (e.g. >400 mg GAE/g DW), which maximize recovery from spent leaves (Harfoush et al., 2024). These comparisons emphasize that while household-style hot brewing efficiently transfers phenolics into the infusion liquor, cold infusions yield residues richer in polyphenols, making them more promising substrates for downstream recovery.

3.3 Total Flavonoid Content (TFC)

The TFC of spent green tea leaves exhibited a clear dependence on brewing temperature, reflecting differences in extraction efficiency and thermal stability of bioactive compounds. Uninfused leaves contained the highest TFC (38.26 mg QE g^{-1} DW), representing the native pool of catechins and flavonols such as quercetin, kaempferol, and rutin. Following cold infusions (20–25 °C, 12 h), residues retained most of this fraction (36.11–37.45 mg QE g^{-1} DW), indicating that low-temperature steeping extracts flavonoids more slowly and leaves the biomass relatively enriched. In contrast, hot infusions (60–100 °C, 3–5 min) caused significant reductions, with residual TFC values declining to 31.27 mg QE g^{-1} DW at 60 °C, 28.93 mg QE g^{-1} DW at 70 °C, and 25.68 mg QE g^{-1} DW at 100 °C. These results are consistent with comparative brewing studies, where hot infusions yield higher flavonoid concentrations in the beverage but leave depleted residues, while cold infusions preserve a greater proportion of flavonoids in the spent matrix (Carloni, Albacete, et al., 2023; Abdeltaif et al., 2018). The mechanism is primarily driven by temperature-enhanced solubilization and diffusion of flavonoids, along with partial epimerization and degradation of heat-labile catechin under boiling conditions. Beyond traditional brewing, recent advances in extraction technologies, such as ultrasound-assisted extraction using natural deep eutectic solvents (NADES), have demonstrated high recovery efficiency of flavonoids from spent tea leaves, further confirming that residues remain a valuable resource (Vo et al., 2024). A broader methodological review also emphasizes the role of process conditions in determining flavonoid yield and stability (Shaukat et al., 2023).

3.4 Condensed Tannins (CT)

Condensed tannins (proanthocyanidins) are polymers of flavan-3-ols that resist hydrolysis and are abundant in many plant foods. They contribute to astringency by binding salivary proteins, while also showing strong antioxidant and antimicrobial properties. Recent studies highlight their role in health promotion, including cardiovascular protection and anti-inflammatory effects (Cosme et al., 2025). In our study, CT levels in spent green tea leaf residues declined with increasing infusion temperature, paralleling the trends observed for TPC and TFC. While uninfused leaves might exhibit a baseline CT content (e.g. ~20–25 mg CE/g DW), residues after cold infusion (20–25 °C, 12 h) tend to retain a significant portion (e.g. 18–22 mg CE/g DW). Hot infusions (60–

100 °C) lead to further reductions (e.g. ~15 mg CE/g at 60 °C, ~12 mg CE/g at 100 °C), due to enhanced leaching and partial breakdown under thermal stress. These patterns are consistent with the understanding that higher temperatures improve solubilization of tannin oligomers and enable diffusion from structural compartments, but also may lead to depolymerization or chemical alteration of larger tannin molecules (Klepacka, 2022; Sezmis et al., 2023).

3.5 Antioxidant Activity and Lipid Peroxidation

Table 3 presents the antioxidant capacity and lipid peroxidation of tea extracts. Antioxidant indices (DPPH, ABTS, FRAP) declined with increasing temperature, while TBARS values rose, indicating enhanced oxidative degradation at higher heat levels.

Table 3 Antioxidant activity (DPPH, ABTS, FRAP) and lipid peroxidation (TBARS) of tea extracts under different temperature conditions

Analyse	Control	Cold 20 °C	Cold 22 °C	Cold 25 °C	Hot 60 °C	Hot 70 °C	Hot 100 °C
DPPH (% RSA)	91.8 ± 2.1^{a}	88.6 ± 2.3^{ab}	87.4 ± 2.0^{b}	85.9 ± 1.9^{b}	78.2 ± 2.4^{c}	73.6 ± 2.1^{d}	68.3 ± 2.5^{e}
ABTS (mg TE g^{-1} MS)	92.35 ± 2.84^{a}	88.17 ± 2.63^{ab}	86.93 ± 2.42^{b}	84.81 ± 2.19^{b}	72.44 ± 2.01^{c}	67.53 ± 1.87^{c}	61.82 ± 1.74^{d}
FRAP (µmol TE g^{-1} MS)	612.4 ± 15.2^{a}	584.7 ± 14.6^{a}	573.5 ± 12.9^{ab}	559.2 ± 11.7^{b}	498.6 ± 13.2^{c}	462.7 ± 12.8^{d}	421.9 ± 11.4^{e}
TBARS (nmol MDA g^{-1} MS)	2.14 ± 0.08^{d}	2.41 ± 0.09^{d}	2.56 ± 0.07^{cd}	2.73 ± 0.11^{c}	3.18 ± 0.12^{b}	3.41 ± 0.10^{b}	3.89 ± 0.14^{a}

Values are mean ± SD (n = 3). Different superscript letters within a row indicate significant differences ($p < 0.05$).

The antioxidant assays collectively highlight a temperature-dependent depletion of radical scavenging and redox buffering capacity in spent tea residues. In the uninfused control, DPPH inhibition reached ~92%, ABTS activity ~92 mg TE g^{-1} DW, and FRAP ~612 µmol TE g^{-1} DW, while TBARS values remained low (~2.1 nmol MDA g^{-1} DW). Cold-infused residues (20–25 °C) maintained values close to the control, with DPPH still >85%, ABTS 85–88 mg TE g^{-1} DW, and FRAP above 550 µmol TE g^{-1} DW. TBARS rose only slightly to 2.4–2.7 nmol MDA g^{-1} DW, suggesting minimal loss of antioxidant buffering. In contrast, hot infusions triggered a graded decline: at 60 °C, DPPH fell to ~78%, ABTS to ~72 mg TE g^{-1} DW, FRAP to ~499 µmol TE g^{-1} DW, and TBARS climbed to ~3.2 nmol MDA g^{-1} DW. The trend intensified at 70 °C and was most pronounced at 100 °C, where DPPH dropped to ~68%, ABTS to ~62 mg TE g^{-1} DW, FRAP to ~422 µmol TE g^{-1} DW, and TBARS nearly doubled compared with control. Expressed as retention rates, cold infusions preserved 91–96% of baseline antioxidant activity, while hot infusions at 100 °C retained only 67–74%, with TBARS increasing by almost 100%. The convergence of results across DPPH, ABTS, FRAP, and TBARS provides robust evidence that higher brewing temperatures deplete antioxidant buffering capacity in the residual biomass, leaving the matrix more vulnerable to oxidative degradation.

The mechanistic drivers of this activity loss can be attributed to both extraction and degradation phenomena. Elevated temperatures enhance diffusion and solvent penetration, accelerating the migration of phenolics and flavonoids into the beverage and thus depleting the residual biomass. Simultaneously, catechin and other thermolabile polyphenols undergo epimerization, polymerization, and oxidative degradation under heat, further reducing antioxidant activity (Deng et al., 2024; Huang et al., 2025). Cold brewing, by contrast, is less aggressive and extracts fewer phenolics, thereby conserving a richer antioxidant pool in the solid fraction (Carloni, Albacete, et al., 2023). In this context, the assay-specific mechanisms further clarify the observed trends: the reductions in

DPPH and ABTS activities correspond to a loss of hydrogen- and electron-donating compounds, the decline in FRAP reflects diminished ferric-reducing capacity due to catechin depletion, and the rise in TBARS indicates weakened lipid-protection capacity as the residue's redox buffering system becomes compromised. The reciprocal rise in TBARS values in hot residues highlights the functional consequences of this depletion: once stripped of redox-active compounds, the matrix becomes less able to buffer lipid peroxidation, consistent with reports in thermally treated tea and plant systems (Fan et al., 2024). These findings illustrate a mechanistic link between consumer brewing practices and the oxidative stability of residues, with direct implications for downstream use.

From a valorization and circularity perspective, this divergence is particularly significant. Cold-brew residues, retaining high levels of radical scavenging and reducing activity, are superior substrates for high-value valorization pathways such as polyphenol recovery, functional food enrichment, nutraceutical development, or even natural preservative formulations. Hot-brew residues, while more depleted, are not without potential: reviews confirm that they still contain extractable phenolics and dietary fibers of functional importance (Çakmak et al., 2024; Shaukat et al., 2023). Their valorization may be optimized through advanced green extraction techniques (e.g., ultrasound-assisted extraction, pressurized solvents, deep natural eutectic solvents) or redirected toward lower-value applications such as compost, biochar, or adsorbents (Negi & Kesari, 2022). The dual fate of residues revealed here underscores that domestic brewing practices not only determine the nutritional composition of the tea beverage but also dictate the biotechnological potential of the waste stream (**Figure 1**). Such insights highlight an often-overlooked leverage point in everyday food preparation with direct relevance to the development of sustainable, circular food systems.

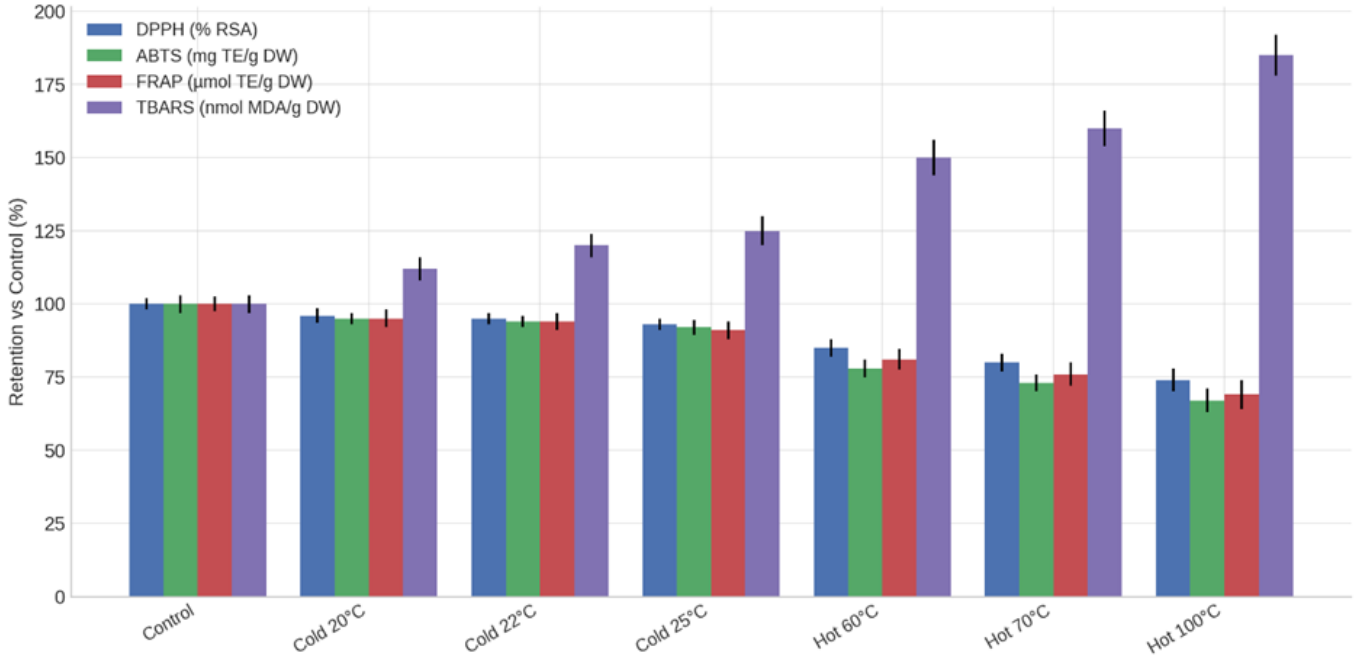


Figure 1 Temperature-dependent changes in antioxidant activity of spent tea residues.

3.6 Antimicrobial Activity

The antimicrobial assays demonstrate a differential inhibitory effect of spent tea residues across microbial strains, with activity modulated strongly by infusion conditions (**Figure 2**). The uninfused control retained the

most potent inhibition across all pathogens, reflecting the full complement of native phenolics, tannins, and catechin. Cold-brew residues (20–25 °C) showed only a modest reduction in inhibition, maintaining much of the antimicrobial profile. By contrast, hot residues (60–100 °C) exhibited progressively diminished activity, with the sharpest loss at 100 °C, underscoring the detrimental impact of heat on phenolic stability and extractability. *S. aureus* was the most sensitive strain across all groups, with inhibition zones in control and cold residues substantially larger than those observed for Gram-negative bacteria. This agrees with reports that Gram-positive species are more susceptible to catechin such as EGCG, which disrupt cytoplasmic membranes and inhibit cell wall synthesis (Feilcke et al., 2023; Liu et al., 2022). Even hot residues retained measurable inhibition, though at significantly reduced levels, indicating partial persistence of active phenolics.

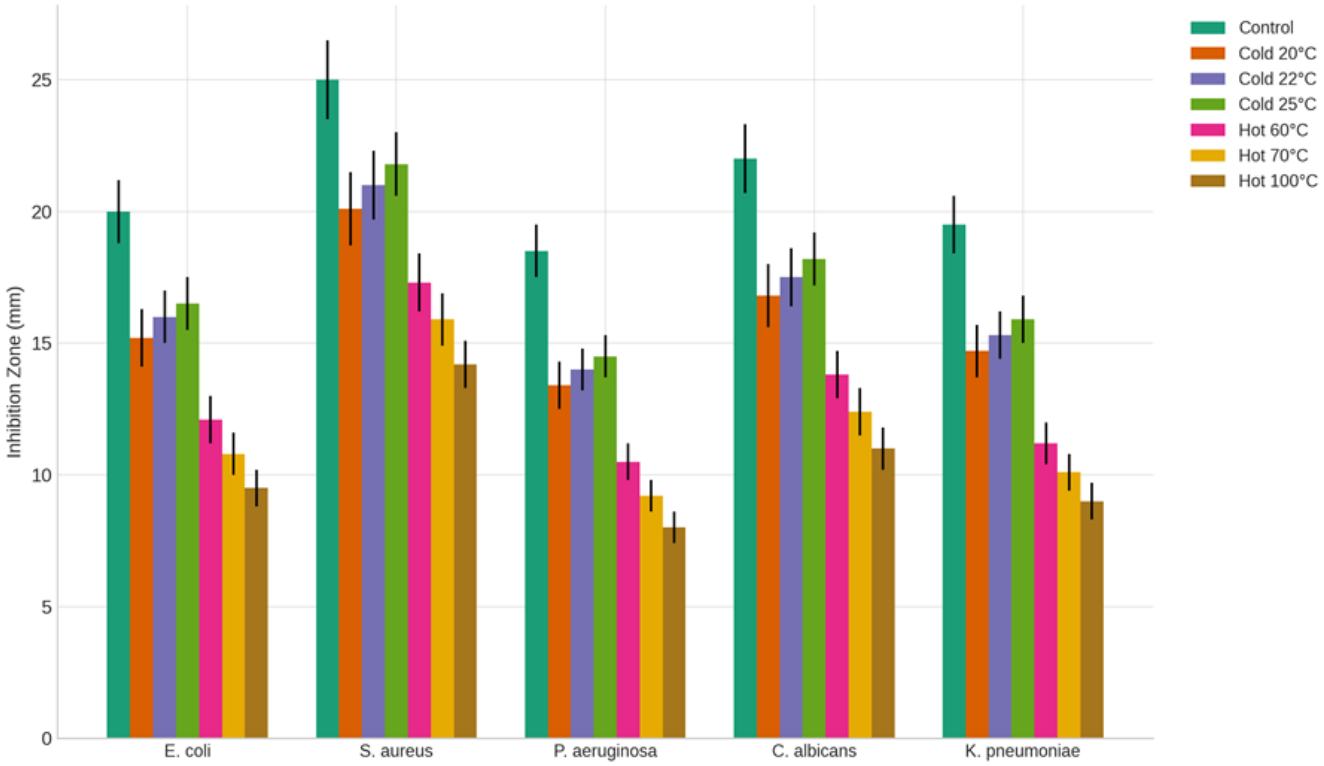


Figure 2 Brewing temperature shapes antimicrobial potential of spent tea residues.

E. coli and *K. pneumoniae* displayed intermediate inhibition profiles: strong in control and cold residues but reduced in hot conditions. The reduced susceptibility of *Enterobacterales* is consistent with their outer membrane barrier and efflux pumps, which restrict catechin penetration (Tallei et al., 2021; Zhang et al., 2021). Nevertheless, cold residues still exhibited inhibitory zones, highlighting that sufficient polyphenolic content remains to exert bacteriostatic effects when heat degradation is avoided. *P. aeruginosa* showed the lowest inhibition across all groups, reflecting its robust intrinsic resistance mechanisms, including multidrug efflux systems and biofilm formation. The steep decline from control to hot residues suggests that any residual effect is highly dependent on intact polyphenols, which are rapidly degraded at higher temperatures. This aligns with studies noting that *Pseudomonas* often requires high concentrations or synergistic combinations to achieve growth inhibition (Liu et al., 2022). *Candida albicans*, representing a fungal pathogen, exhibited intermediate inhibition comparable to Gram-negative bacteria, with cold residues maintaining clear activity while hot residues (particularly 100 °C) lost much of their efficacy. This agrees with recent findings that tea polyphenols can inhibit fungal virulence factors, such as hyphal growth and biofilm formation, though activity diminishes when

polyphenols are degraded or leached into the infusion (Urme et al., 2024). Taken together, these results highlight two converging trends: taxonomic sensitivity hierarchy — *S. aureus* > *E. coli* ≈ *K. pneumoniae* > *P. aeruginosa*, with *C. albicans* showing intermediate responses. Additionally, temperature gradient effect — control ≈ cold > hot, reflecting progressive phenolic depletion and structural modification under thermal stress.

These findings align with broader valorization literature showing that even after hot brewing, residues retain extractable bioactive fractions, but their yield and potency depend strongly on extraction conditions (Çakmak et al., 2024; Shaukat et al., 2023). From a circular bioeconomy perspective, cold-brew residues appear most suitable for high-value antimicrobial applications (e.g. functional food fortification, antimicrobial packaging, or biopreservatives). Hot-brew residues, while diminished, could be revalorized through green extraction technologies such as ultrasound-assisted extraction or encapsulation strategies to recover usable phenolics (Fabrikov et al., 2024; Fan et al., 2024).

4 Conclusion

This study demonstrates that household tea waste leaves, often discarded as a byproduct of daily consumption, retain substantial nutritional and functional potential that is strongly modulated by infusion conditions. Cold-brew residues preserved higher levels of protein, dietary fiber, phenols, flavonoids, tannins, and antioxidant activity, alongside stronger antimicrobial efficacy, compared with hot-brew residues where thermal degradation and compound leaching were more pronounced. These findings establish a mechanistic link between consumer brewing practices and the downstream valorization capacity of spent leaves. From a sustainability perspective, tea residues represent a versatile biomass stream with promising applications in functional foods, nutraceuticals, cosmetics, and bio-based materials. Cold-brew residues emerge as superior candidates for high-value applications such as antioxidant recovery or incorporation into fiber- and protein-enriched foods, while hot-brew residues, though depleted, remain viable for transformation through advanced green extraction or circular uses such as compost, adsorbents, or biochar. By reframing tea residues as a nutrient-rich resource rather than waste, this work highlights an overlooked leverage point in household food practices that directly supports circular bioeconomy objectives. Integrating tea-waste valorization into food and bioproduct systems not only reduces environmental burdens but also creates new opportunities for innovation, value creation, and sustainable food futures.

Author contributions

Said Dahmouni: Conceptualization, methodology, investigation, writing—original draft, writing—review and editing. **Zineb Bengharbi**: Conceptualization, methodology, investigation, writing—reviewing and editing, validation, project administration, and supervision. **Djilali Benabdelmoumene**: Data curation, formal analysis, investigation, writing—review and editing, project administration, and supervision. **Salim Nebbache**: Investigation, formal Analysis, and validation. **Wasim S. M. Qadi:** Conceptualization, methodology, writing—review and editing. **Ahmed Mediani:** Data curation, methodology, investigation. All authors have read and approved the final version of the manuscript.

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All authors contributed to the final manuscript, reviewed its content critically, and approved it for submission.

Conflict of Interest

The authors declare that there are no financial or commercial conflicts of interest in connection with this research.

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