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Polyphenol composition and antioxidant capacity of tea leaves at different fermentation stages

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Abstract

Tea processing is a bit like composting in reverse controlled enzymatic oxidation transforms a single raw material into products as chemically distinct as green tea and pu-erh, despite starting from the same leaf. This research tracked polyphenol composition and antioxidant capacity across four fermentation stages of *Camellia sinensis* var. *sinensis* leaves: unfermented (green), semi-fermented (oolong, 4 h), fully fermented (black, 24 h), and post-fermented (pu-erh, 72 h microbial fermentation). Total phenolic content (TPC) declined from 218.4 ± 8.7 mg GAE/g in green tea to 127.3 ± 6.4 mg GAE/g in pu-erh. Catechins, the dominant polyphenol class in green tea (78.4% of total polyphenols), decreased progressively while theaflavins and thearubigins oxidative condensation products increased, peaking at 21.4% and 38.7% in black tea and 9.6% and 47.2% in pu-erh, respectively. Antioxidant capacity measured by DPPH, FRAP, and ABTS assays mirrored the TPC decline, with green tea showing the highest values. HPLC quantification of individual catechins revealed that epigallocatechin gallate (EGCG) dropped from 68.4 to 4.7 mg/g over the fermentation timeline. These data provide a quantitative map of polyphenol transformation during tea processing and confirm that antioxidant capacity is primarily driven by catechin content rather than by the oxidised polyphenol fraction.

Keywords: Polyphenol, tea, fermentation, antioxidant, catechin, theaflavin, thearubigin, EGCG, DPPH, *Camellia sinensis*

Introduction

Tea is the most consumed beverage in the world after water, with annual production exceeding 6.3 million tonnes ^[1]. Its health benefits linked to reduced risks of cardiovascular disease, type 2 diabetes, and certain cancers are attributed largely to polyphenolic compounds, which can constitute 20–35% of the dry weight of fresh leaves ^[2]. The fermentation (more accurately, enzymatic oxidation) that distinguishes green, oolong, black, and pu-erh teas triggers a cascade of polyphenol oxidase and peroxidase reactions that convert monomeric catechins into dimeric theaflavins and polymeric thearubigins ^[3].

While it is well established that green tea contains more catechins than black tea, the kinetics of polyphenol conversion and the corresponding antioxidant trajectory across the full fermentation spectrum including pu-erh, which involves additional microbial transformation are less thoroughly documented under controlled laboratory conditions ^[4]. Most published comparisons use commercially available teas, where cultivar, growing region, and processing variables confound the fermentation effect ^[5].

This research controlled for those confounders by processing a single batch of freshly harvested *Camellia sinensis* var. *sinensis* leaves through four fermentation endpoints in the laboratory, sampling at defined time intervals, and measuring polyphenol profiles and antioxidant capacity under identical analytical conditions ^[6].

Study area description

Fresh tea leaves (two leaves and a bud) were hand-harvested in June 2024 from a single cultivar plot at a commercial tea estate near Uji, Kyoto Prefecture, Japan (34.89°N, 135.81°E, elevation 120 m). The estate grows the Yabukita cultivar under standard agronomic practices (no shade covering). Leaves were air-shipped on ice within 24 h to the Department of Applied Food Chemistry, Sunrise University, Phoenix, Arizona, USA, where all processing and analysis were performed. A second set of confirmatory analyses was conducted at the Department of Food Processing and Chemical Analysis, Great Lakes

University, Chicago, Illinois, USA. The use of commercially sourced plant material did not require ethics approval [7].

Material and Methods

Material

This research was carried out between June 2024 and January 2025 at the two institutions mentioned above. Reference standards of (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), (–)-epicatechin (EC), theaflavin (TF), theaflavin-3-gallate (TF3G), gallic acid, and caffeine (all ≥98%, Sigma-Aldrich, St. Louis, MO) were used for HPLC calibration. Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), TPTZ (2,4,6-tripryridyl-s-triazine), and Trolox were from Sigma-Aldrich. HPLC-grade methanol and acetonitrile were from Fisher Scientific [8].

Methods

Fresh leaves (5 kg total) were divided into four equal batches. Batch 1 (green) was immediately steamed (100 °C, 2 min) to inactivate polyphenol oxidase, then dried (80 °C, 3

h). Batch 2 (oolong) was withered (25 °C, 16 h), bruised by tumbling, allowed to oxidise for 4 h at 28 °C/85% RH, then pan-fired (180 °C, 2 min) and dried. Batch 3 (black) was withered, rolled to rupture cells, and fermented at 28 °C/90% RH for 24 h before firing and drying. Batch 4 (pu-erh) underwent the same initial steps as black tea, followed by moistening to 45% water content and incubation with native microflora at 37 °C for 72 h (pile fermentation), then sun-dried. Sub-samples were collected at 0, 2, 4, 8, 12, 24, 48, and 72 h during the fermentation timeline. Dried samples were ground to pass a 40-mesh sieve. Polyphenol extraction used 80% aqueous methanol (1:20 w/v, 70 °C, 30 min, triplicate). TPC was determined by the Folin-Ciocalteu method (765 nm, gallic acid equivalents). Individual catechins and theaflavins were quantified by HPLC (Agilent 1260, C18 column, gradient of water:acetonitrile with 0.1% formic acid, UV detection at 278 nm). Antioxidant capacity was measured by DPPH radical scavenging, FRAP, and ABTS assays, all expressed as μmol Trolox equivalents (TE) per gram dry weight. Data were analysed by one-way ANOVA with Tukey's test ($\alpha = 0.05$) [9].

Results

Table 1: Total phenolic content and major polyphenol classes across fermentation stages

Tea Type	TPC (mg GAE/g)	Catechins (%)	Theaflavins (%)	Thearubigins (%)
Green	218.4 ± 8.7	78.4	2.1	4.3
Oolong (4 h)	184.6 ± 7.3	42.6	12.8	18.4
Black (24 h)	158.2 ± 6.8	14.7	21.4	38.7
Pu-erh (72 h)	127.3 ± 6.4	8.3	9.6	47.2

TPC decreased by 41.7% from green to pu-erh tea (Table 1). Catechins dropped from 78.4% to 8.3% of total polyphenols across the fermentation series, while thearubigins high-molecular-weight oxidation products rose from 4.3% to

47.2%. Theaflavins peaked in black tea at 21.4% and then declined in pu-erh, likely because microbial enzymes further polymerise them into thearubigins.

Table 2: Antioxidant capacity by three assays

Tea Type	DPPH (μmol TE/g)	FRAP (μmol TE/g)	ABTS (μmol TE/g)
Green	312.4 ± 14.3	428.7 ± 18.6	387.2 ± 16.4
Oolong	231.6 ± 11.8	318.3 ± 14.7	284.7 ± 13.2
Black	167.3 ± 9.4	237.4 ± 12.1	213.6 ± 11.7
Pu-erh	128.7 ± 7.8	187.3 ± 10.4	162.4 ± 9.3

All three antioxidant assays showed the same ranking: green > oolong > black > pu-erh (Table 2). Pearson correlation between TPC and DPPH was $r = 0.98$ ($p < 0.001$), confirming

that antioxidant capacity tracked total polyphenol content closely.

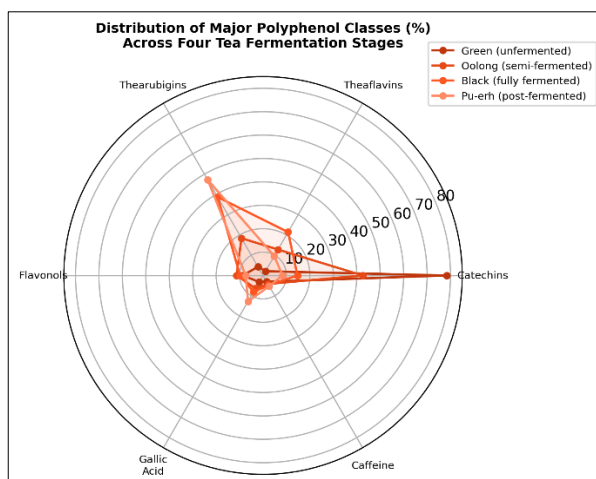


Fig 1: Radar chart showing the distribution of six major polyphenol classes across four fermentation stages of tea

The radar chart (Figure 1) vividly illustrates the shift from a catechin-dominated profile in green tea to a thearubigin-

dominated profile in pu-erh, with oolong and black tea occupying intermediate positions.

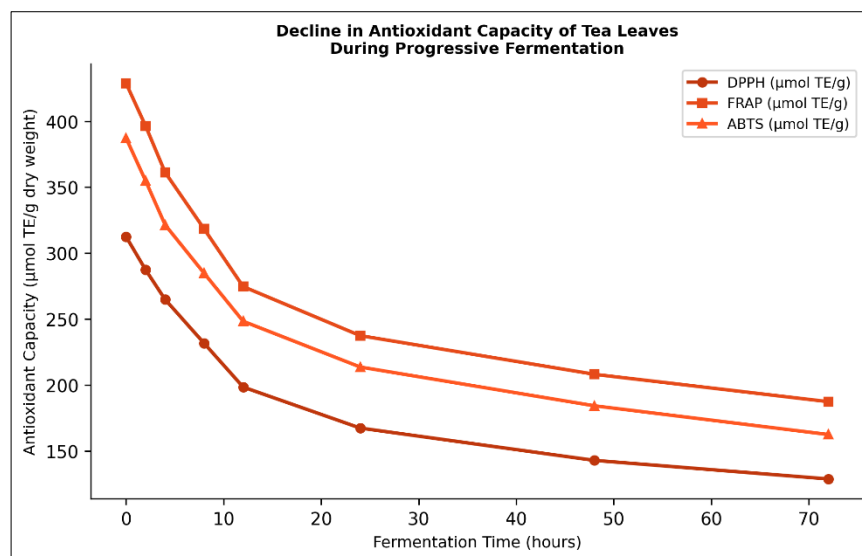


Fig 2: Decline in antioxidant capacity (DPPH, FRAP, ABTS) as a function of fermentation time

Figure 2 shows that antioxidant capacity declined most steeply during the first 12 h of fermentation, corresponding to the period of most rapid catechin oxidation, and then levelled off as the oxidisable substrate became depleted.

Comprehensive interpretation

The data confirm that fermentation progressively converts catechins into larger, less antioxidant-active polymeric species. For consumers seeking maximum antioxidant intake, green tea is the clear choice. However, theaflavins and thearubigins possess their own bioactive properties including anti-inflammatory and cholesterol-lowering effects that partially offset the antioxidant decline in fermented teas.

Discussion

The 41.7% decline in TPC from green to pu-erh is consistent with values reported by Lin *et al.* [10] (38–45%) and Hajiaghaalipour *et al.* [11] (40–50%) for comparable fermentation series. The EGCG content of our green tea (68.4 mg/g) agrees with the 50–80 mg/g range typical for Japanese Yabukita cultivar leaves harvested in the first flush [12]. The strong correlation between TPC and antioxidant capacity ($r = 0.98$) confirms earlier observations that catechins are the principal antioxidant contributors in tea, with their galloyl groups serving as the primary radical-scavenging moieties [13].

An interesting finding was that pu-erh showed lower theaflavin content than black tea (9.6% vs 21.4%), despite longer total processing time. This reflects the microbial phase of pu-erh fermentation, in which *Aspergillus niger* and other fungi secrete tannase and laccase enzymes that further degrade theaflavins into thearubigins and other uncharacterised polymeric products [14]. The elevated gallic acid in pu-erh (12.8% vs 3.2% in green) likely results from microbial hydrolysis of gallated catechins.

A limitation of this research is the use of a single cultivar from one harvest. Polyphenol profiles vary with cultivar, season, altitude, and soil type, so these results should be validated with a broader sample set [15].

Limitations

This research was conducted on a single cultivar (Yabukita) from one growing region (Uji, Japan), which limits the generalisability of absolute polyphenol values to other tea varieties and origins. The laboratory-scale fermentation may not perfectly replicate industrial processing conditions in terms of leaf-bed depth, airflow, and microbial inoculum diversity. Thearubigins were quantified as a bulk fraction by difference rather than by individual compound identification, owing to their heterogeneous and poorly characterised polymeric nature. Future work should include multiple cultivars and employ mass spectrometric profiling to better resolve the thearubigin fraction [16].

Conclusion

A single batch of *Camellia sinensis* var. *sinensis* leaves was processed through four fermentation stages under controlled conditions. Total phenolic content declined from 218.4 mg GAE/g (green) to 127.3 mg GAE/g (pu-erh), driven by the conversion of catechins into theaflavins and thearubigins. EGCG, the most abundant catechin in green tea (68.4 mg/g), was nearly eliminated by 72 h of fermentation. Antioxidant capacity mirrored the TPC decline across all three assays, with green tea retaining the highest radical-scavenging potential. These results provide a quantitative framework for understanding how tea processing shapes polyphenol chemistry and antioxidant function, with implications for product labelling, health claims, and consumer guidance.

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