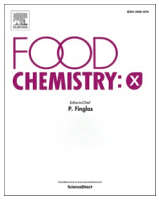
Food Chemistry: X 33 (2026) 103473

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Comparative evaluation of high-pressure processing and conventional pasteurization in cold brew green tea: *In vitro* digestibility, bioavailability, and nutrient stability☆

Natthawuddhi Donlao [a,b,*], Chhoryi Sim [c], Jaspreet Singh [d,e], Lovedeep Kaur [d,e], Jinhu Tian [f], Suphat Phongthai [g], Yardfon Tanongkankit [h], Utthapon Issara [i], Chanthima Phungamngoen [j], Wirongrong Tongdeesoontorn [a], Saranchanok Wonglek [a]

[a] *Research Center of Innovative Food Packaging and Biomaterials Unit, Mae Fah Luang University, 333 Moo 1 Tasud, Chiang Rai 57100, Thailand*
[b] *Tea and Coffee Institute, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[c] *Department of Bio-Engineering, Faculty of Engineering, Royal University of Phnom Penh (Campus 1), Phnom Penh, Cambodia*
[d] *School of Food Technology and Natural Sciences, Massey University, Palmerston North 4442, New Zealand*
[e] *Riddet Institute, Massey University, Palmerston North 4442, New Zealand*
[f] *College of Biosystems Engineering and Food Science, National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing, Integrated Research Base of Southern Fruit and Vegetable Preservation Technology, Zhejiang International Scientific and Technological Cooperation Base of Health Food Manufacturing and Quality Control, Fuli Institute of Food Science, Zhejiang University, Hangzhou, China*
[g] *Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand*
[h] *Department of Agricultural and Food Engineering, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290, Thailand*
[i] *Division of Food Science and Technology Management, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathum Thani 12110, Thailand*
[j] *Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand*

A R T I C L E I N F O

Keywords:
High-pressure processing
Cold-brew tea
Catechins
Microbial safety
Antioxidants
In vitro digestion

A B S T R A C T

High-pressure processing (HPP) is a non-thermal alternative to thermal pasteurization (Ps) for preserving cold-brewed green tea. This study compared HPP (300, 450, and 600 MPa for 5 and 10 min) with Ps for quality, microbial safety, and *in vitro* digestive stability. Moderate HPP treatments preserved color, ascorbic acid, and catechins effectively. HPP retained more non-galloylated catechins (GC, EGC, and EC) than Ps, which could reduce bitterness and astringency, though formal sensory evaluation is needed to confirm this. Among the conditions, 600 MPa for 5 min was optimal for overall quality, while 600 MPa for 10 min ensured maximal microbial inactivation. During digestion, catechins, total phenolic content, and antioxidant activity (DPPH, FRAP) decreased significantly ($p < 0.05$) but retained >70 %, demonstrating satisfactory stability. Overall, HPP is an effective method to maintain both the nutritional quality and microbial safety of cold-brewed green tea.

1. Introduction

Global tea production has seen a steady annual growth of 3.2 % over the past decade, reaching 6.7 million metric tons in 2022. Green tea, an unfermented variety, ranks as the second most consumed type of tea worldwide, trailing only black tea. The green tea market is expanding at a remarkable rate of 4.9 % annually—almost double the growth rate of black tea—reflecting an increasing consumer demand (FAO, 2024). This surge in popularity is driven by the health benefits attributed to green tea, which include promoting cardiovascular health, reducing cholesterol, alleviating anxiety, preventing arthritis, and enhancing immune function (Tallei et al., 2021).

Green tea's bioactive compounds, particularly catechins, largely contribute to its health-promoting properties. These polyphenolic antioxidants, which include (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epicatechin

☆ This article is part of a Special issue entitled: 'In vitro digestion' published in Food Chemistry: X.

* Corresponding author at: Research Center of Innovative Food Packaging and Biomaterials Unit, Mae Fah Luang University, 333 Moo 1 Tasud, Chiang Rai 57100, Thailand.
E-mail address: natthawuddhi.don@mfu.ac.th (N. Donlao).

https://doi.org/10.1016/j.fochx.2025.103473
Received 27 May 2025; Received in revised form 13 December 2025; Accepted 29 December 2025
Available online 30 December 2025

(EC), play a key role in the tea's beneficial effects. Among these, EGCG is the most abundant and serves as a quality indicator for green tea. In addition to catechins, green tea contains other polyphenols such as gallic acid, quercetin, kaempferol, and myricetin, which contribute to its antioxidant capacity and health benefits (Ananingsih, Sharma, & Zhou, 2013).

Traditionally, green tea is prepared by steeping tea leaves in water at temperatures of 80–90 °C, which extracts both flavor and bioactive components. However, with the growing demand for green tea, alternative methods such as cold brewing have gained popularity. Cold brewing, which involves steeping tea leaves in water at temperatures ranging from 4 to 25 °C for an extended period, results in a smoother taste, reduced bitterness, lower caffeine content, and better preservation of sensory properties (Lin, Yang, Hsieh, Liu, & Mau, 2014). This method also retains more bioactive compounds due to the absence of heat. However, the longer steeping times associated with cold brewing increase the risk of microbial contamination, necessitating effective preservation methods (Song et al., 2021).

Pasteurization, a common method for food safety, reduces enzymatic activity and eliminates pathogens but often results in the degradation of flavor, color, and nutritional content. In contrast, High Hydrostatic Pressure (HPP), also known as Ultra-High Pressure (UHP), has emerged as a promising non-thermal alternative to traditional pasteurization. HPP has been shown to improve the extraction of bioactive compounds, enhance microbiological safety, and preserve the quality of tea extracts while reducing flavor deterioration and minimizing environmental impact compared to thermal pasteurization (Šeremet et al., 2021; Song et al., 2021).

Despite the known benefits of HPP, research focusing specifically on its application to cold-brewed green tea remains limited. Therefore, the objective of this study was twofold: to identify the optimal HPP pressure and duration for processing cold-brewed green tea and to assess the impacts of HPP on the physicochemical properties, antioxidant activity, and bioactive compound content of the tea. Sensory attributes were evaluated using an electronic nose (*E*-nose), providing objective and reproducible analysis. Additionally, the stability of bioactive compounds was assessed through a two-stage *in vitro* gastrointestinal digestion model. This study explores the potential of HPP to enhance the quality and safety of cold-brewed green tea, enabling producers to create a preservative-free, additive-free product that appeals to health-conscious consumers. Ultimately, this approach provides a sustainable solution for meeting stringent food safety and quality standards without compromising the taste or nutritional value of the product.

2. Materials and methods

2.1. Chemicals

Acetonitrile, sodium hydroxide, and ethanol were obtained from RCI Labscan Chemicals (Bangkok, Thailand). Folin-Ciocalteu's phenol reagent and ferric chloride were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Trifluoroacetic acid for HPLC, gallic acid monohydrate, 2,2-diphenyl-1-picrylhydrazyl, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), amino acid standard, catechin standard, pepsin from porcine gastric mucosa (P7000, ≥250 units/mg solid), pancreatin from porcine pancreas (4xUSP), amyloglucosidase (3260 U/mL), and potassium dichromate solution were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade. Hydrochloric acid, sodium carbonate, sodium acetate, potassium chloride, and ferrous sulfate were purchased from Qrec (New Zealand). Deionized water was obtained using an in-house Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Tea samples

Sencha green tea (*Camellia sinensis* cv. Jin Xuan) was provided by Boonrawd Farm Co., Ltd., and Choke Chamroen Tea Co., Ltd., located in Chiang Rai, Thailand. The tea leaves were harvested in April 2024 using the fine-plucking method, which involves selecting shoots with two leaves and a bud. The tea leaves were processed following a traditional Japanese method, which includes the following steps: withering to preserve freshness, rolling, steaming, and drying.

2.3. Preparation of tea infusions

2.3.1. Hot and cold brew tea infusion

Hot brew tea (HB) and cold brew tea (CB) infusions were prepared according to Chiang, Tsou, Chang, and Chen (2020) with some modifications. For the hot brew tea infusion, 20 g of tea leaves were placed in 1 L of boiling water (20:1, *w/v*) and steeped for 5 min. For the cold brew tea infusion, 20 g of tea leaves were placed in 1 L of water (20:1, w/v) and steeped in a cold room maintained at 4 °C for 12 h. Both infusions were filtered through cheesecloth and subsequently packed in PET bottles prior to pasteurization, as described in Section 2.2.2.

2.3.2. Process of pasteurization and high-pressure processing (HPP)

For conventional pasteurization (Ps), bottled hot-brew and cold-brew green tea infusions were immersed in a water bath (Memmert, Schwabach, Germany) at 65 °C for 30 min (Bellumori et al., 2021). These samples were labeled HB + Ps and CB + Ps, respectively.

Bottled cold-brew green tea infusions (80 mL) underwent high-pressure processing (HPP) using a high-pressure generator (Bao Tou KeFa, BTKF High Pressure Technology Co., Ltd., Mongolia, China). The system automatically adjusts the pressurization and depressurization rates according to the machine's specifications. Pressurization typically occurs at a controlled rate of approximately 1–2 MPa/s, and depressurization is similarly managed to avoid mechanical stress on the material. HPP was applied at three operating pressures: 300, 450, and 600 MPa, with two different holding times: 300 s and 600 s at room temperature. These HPP-treated samples were designated as CB300:5, CB300:10, CB450:5, CB450:10, CB600:5, and CB600:10. Untreated cold-brewed samples (CB), which were cold-brewed without pasteurization or HPP treatment, were included as controls.

All processed tea samples were refrigerated at 4 °C and filtered using Whatman No. 1 filter paper (GE Healthcare UK, Buckinghamshire, UK) prior to analysis. The abbreviations used in this study are provided in Table S1 (Supplementary Material).

2.4. Determination of physical properties

2.4.1. Color measurement

The color of the filtered tea infusions was measured using a handheld spectrophotometer (CM-600d, Konica Minolta, Tokyo, Japan) based on the CIE L*, a*, b* color system. Measurements were taken in triplicate. The L* value represents lightness, ranging from 0 (darkest black) to 100 (brightest white). The a* value indicates redness (positive values) and greenness (negative values), while the b* value represents yellowness (positive values) and blueness (negative values). The total color change (ΔE) was calculated using the following eq. (1), as described by Yeo et al. (2024):

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \quad (1)$$

2.4.2. Determination of turbidity

Turbidity was assessed by measuring the light transmittance of the tea infusion at 640 nm using a UV-VIS spectrophotometer (V-630, Jasco, Tokyo, Japan). The spectrophotometer was equipped with both a deuterium lamp and a tungsten halogen lamp, and it was calibrated with

a potassium dichromate solution prior to testing.

2.4.3. pH and total soluble solid (TSS)

The pH of the filtered tea infusion was measured using a pH meter (FiveEasy Plus pH meter F20, Mettler Toledo, Switzerland). The total soluble solids (TSS) content was determined using a digital refractometer (PAL-1, Atago, Japan) at room temperature.

2.5. Determination of chlorophyll content

Chlorophyll content was determined using a colorimetric method adapted from Huang, Sheng, Yang, and Hu (2007) with minor modifications. Dried green tea leaves were ground to a fine powder using a porcelain mortar and pestle. For chlorophyll extraction from the leaves, approximately 0.1 g of powder was mixed with 25 mL of 80 % (*v*/v) acetone and homogenized using a vortex mixer (Vortex Mixer Genie 2, Scientific Industries, Bohemia, NY, USA). For green tea infusions, 1 mL of filtered tea infusion was diluted with 4 mL of 100 % acetone and homogenized in the same manner. All extracts were filtered through Whatman No. 1 filter paper (GE Healthcare, Kent, UK) prior to analysis.

Chlorophyll concentrations were measured using a Thermo Scientific GENESYS 10S UV–Vis spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a standard tungsten–halogen light source. Absorbance was recorded at 663 nm for chlorophyll *a* and 645 nm for chlorophyll *b*. Concentrations were calculated using the following formulas:

$$\text{Content of chlorophyll } a \text{ (mg/l)} = (12.7 \times A_{663}) - (2.95 \times A_{645}) \quad (2)$$

$$\text{Content of chlorophyll } b \text{ (mg/l)} = (22.9 \times A_{645}) - (4.67 \times A_{633}) \quad (3)$$

Absorbance values were corrected for potential interferences from the sample matrix by subtracting the absorbance of the respective solvent blank.

2.6. Determination of ascorbic acid (AA) content

Ascorbic acid (AA) content was determined using the method outlined by AOAC (2016). For analysis, 2 mL of filtered tea infusion was mixed with 5 mL of a metaphosphoric acid-acetic acid solution in a 50-mL Erlenmeyer flask. The mixture was then titrated with an indophenol dye solution until a light rose-pink color persisted for more than 5 s. The concentration of AA was calculated using the following equation:

$$\text{mg}\frac{\text{AA}}{\text{mL}} = (X - B) \times (F/E) \times (V/Y) \quad (4)$$

where X = mL for sample titration, B = average mL for sample blank titration, F = titer of dye, E = mL assayed, V = volume of initial assay solution, and Y = volume of sample aliquot titrated.

2.7. Determination of catechins and caffeine profile

The determination of catechins and caffeine in green tea was performed using a high-performance liquid chromatography (HPLC) system equipped with a photodiode array (PDA) detector (Waters® 2996, Waters Corporation). Tea samples were prepared by filtering methanol and aqueous extracts through a 0.45 μm nylon syringe filter (Whatman International Ltd.). A 10 μL aliquot of each filtered tea extract was injected into a GRACE Platinum C18-EPS column (3 μm, 53 mm × 7 mm, W. R. Grace & Co.) maintained at 30 °C. The mobile phase consisted of Solution A (0.05 % trifluoroacetic acid in water) and Solution B (99.9 % acetonitrile), both filtered through a 0.45 μm cellulose acetate filter. The chromatographic conditions were set to isocratic elution with 87 % Solution A and 13 % Solution B at a flow rate of 2.0 mL/min for 10 min. The PDA detector was set at 210 nm to monitor the eluent. Catechin and caffeine standards were prepared at concentrations ranging from 0.20 to 100.00 μg/mL across 9 levels, with duplicate injections for each sample. Quantification was performed using the external standard method by comparing retention times and spectral data with the valid standards. The method exhibited good linearity ($R^2 > 0.99$) and acceptable precision, as indicated by relative standard deviations (RSD%) in repeated injections. Catechins and caffeine were quantified in mg/mL of tea infusion. The standard curve range and recovery were also assessed to ensure the accuracy of the analysis.

2.8. Determination of total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu assay (Wonglek et al., 2024). Add 500 μL of sample to test tubes, then add 2.5 mL of 10 % Folin-Ciocalteu's reagent and 2 mL of 7.5 % sodium carbonate. After mixing with a vortex mixer, the mixture was left at room temperature for 1 h. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer (V-630, Jasco, Tokyo, Japan) and distilled water as the blank. TPC was mg gallic acid equivalent (GAE) per mL tea infusion.

2.9. Determination of total antioxidant activity

2.9.1. 1,1 -diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay

The DPPH free-radical scavenging assay was performed following the method of Wonglek et al. (2024) with slight modifications. This assay measures antioxidant free radical scavenging activity. A 50 μL diluted sample was combined with 1.95 mL of a 60 μM DPPH solution. The mixture was thoroughly vortexed and then kept in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a spectrophotometer. Radical scavenging activity was expressed in μmol Trolox equivalent (TE) per mL of tea infusion.

2.9.2. Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out following the method of Wonglek et al. (2024) with minor modifications. This assay evaluates the ability of antioxidants to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. The FRAP reagent solution was prepared by mixing 300 mM acetate buffer (adjusted to pH 3.60), 10 mM 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 ratio. A 400 μL sample was combined with 2.6 mL of FRAP reagent. The mixture was stirred using a vortex mixer and incubated in a temperature-controlled water bath at 37 °C for 30 min in the dark. The absorbance was measured at 595 nm using a spectrophotometer. FRAP values were expressed in μmol $FeSO_4$ equivalents per mL of tea infusion.

2.10. Determination of amino acid profile and composition

The amino acid profile of green tea infusions was determined using a liquid chromatography-triple quadrupole mass spectrometer (LC-QqQ-MS) system (SHIMADZU LCMS-8060) equipped with a binary pump and autosampler. The sampling speed was set at 5.0 μL/s, and each sample injection volume was 1 μL. The mobile phase consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B).

For amino acid analysis, 50 mg of dried tea sample was hydrolyzed with 8 mL of 6 M HCl at 110 °C for 22 h. After cooling, 4.8 mL of 10 M NaOH was added to neutralize the solution, and the final volume was adjusted to 25 mL with distilled water. The mixture was filtered and centrifuged at 10,000 ×*g* for 10 min. The supernatants were then diluted 25× and 50× with 0.1 N HCl, followed by filtration through a 0.2 μm syringe filter.

A 1 μL aliquot of the prepared sample was injected into the LC-QqQ-MS for quantification. The results were expressed as a percentage by weight (*w*/w) on a dry weight basis (DW). All analyses were performed in triplicate to ensure precision and reproducibility.

2.11. Compound light microscope

A 0.5 mL aliquot of green tea infusion was placed onto a microscope glass slide and covered with a coverslip. The slide was then examined using a compound light microscope (CX22LED, Olympus, Tokyo, Japan) at magnifications ranging from 10× to 100×. Immersion oil was used with the 100× objective lens.

2.12. Electronic nose (E-nose) analysis

Electronic nose measurements were performed using the Mobilis Automata E-nose (MUI Robotics, MUI Robotics Company Ltd., Nontaburi, Thailand) equipped with a 16-sensor Metal Oxide Semiconductor (MOS) array. Sensor IDs and their target volatile groups are provided in supplementary Table S2 (Supplementary Material).

For each analysis, 30 mL of green tea infusion was transferred into a 100 mL glass media bottle and placed on the designated sample holder. The system included two positions: one for the sample bottle and one for a blank reference bottle. Both bottles were fitted with airtight caps connected to the E-nose through Teflon tubing to ensure stable headspace delivery.

Prior to measurement, each sample underwent headspace equilibration at 50 °C, a temperature selected to promote consistent release of volatile compounds and enhance sensor responsiveness (Huang, Doh, & Bae, 2021). The instrumental protocol consisted of three measurement cycles per sample, with each cycle lasting 5 min, resulting in a total acquisition time of 15 min per sample.

A blank run (clean bottle) was performed after every sample to allow the system to purge residual volatiles and recalibrate the baseline response. All samples were analyzed in triplicate.

Sensor output signals were exported for multivariate analysis. Data preprocessing included baseline correction and normalization to minimize drift and sample-to-sample variability. Principal Component Analysis (PCA) was conducted using the normalized sensor response values from all 16 sensors to explore the discrimination patterns among samples.

2.13. Microbial analysis

Microbial analysis of tea infusions was performed to determine total aerobic bacteria, yeast and mold counts, and the presence of *E. coli* and coliforms, following AOAC International (2016) guidelines. A 1.0 mL aliquot of each tea infusion was plated in duplicate onto 3 M™ Petrifilm™ Aerobic Count Plates for total plate count (TPC), 3 M™ Petrifilm™ Rapid Yeast and Mold Count Plates for yeast and mold enumeration, and 3 M™ Petrifilm™ *E. coli*/Coliform Count Plates for *E. coli* and coliform detection. Plates were incubated under the following conditions: TPC at 35 °C for 48 ± 2 h, yeast and mold at 25 °C for 48–72 h, and *E. coli*/coliforms at 35 °C for 24–48 h. Colonies were enumerated visually according to the manufacturer's instructions. The limits of detection (LOD) were 10 CFU/mL for TPC and yeast/mold and 1 CFU/mL for *E. coli*/coliforms. Counts below the LOD were reported as <LOD and treated as half of the LOD (0.5 × LOD) for statistical analysis and plotting, in accordance with standard microbiological practice. All assays were performed in duplicate to ensure accuracy and reproducibility.

2.14. In vitro gastrointestinal digestion

A static *in vitro* gastrointestinal digestion model, adapted from Wonglek et al. (2024), was employed to evaluate the stability of total phenolic content, antioxidant activity, and catechins in cold-brew tea infusions. The experiment was conducted in a laboratory borosilicate glass reactor (manufacturer and model not specified) connected to a Memmert water bath (Memmert, Schwabach, Germany) maintained at 37 °C, with continuous stirring at 200 rpm using a magnetic stirrer. A 170 mL aliquot of tea infusion was added to the reactor along with a magnetic stirring bar to ensure homogeneous mixing.

2.14.1. Gastric phase

To simulate gastric digestion, the pH of the sample was adjusted to 2.00 using 1 M HCl. 19 mL of pepsin solution was then added to initiate the gastric phase. The pepsin solution was prepared by dissolving 0.24 g of pepsin from pig stomachs (Sigma-Aldrich, St. Louis, MO, USA; activity 800–2500 U/mg protein) in 50 mL of gastric fluid buffer (0.03 M NaCl, 0.075 M KCl, pH 1.20). The pH of the mixture was immediately readjusted to 1.20 ± 0.01 and maintained for 30 min at 37 °C.

2.14.2. Intestinal phase

The gastric phase was terminated by adjusting the pH to 6.00, effectively deactivating pepsin. The intestinal phase was initiated by adding 23 mL of intestinal enzyme solution, prepared by dissolving 0.2 g pancreatin (Sigma-Aldrich, swine pancreas) and 4 mL amyloglucosidase (Megazyme, Co. Wicklow, Ireland) in 25 mL intestinal fluid buffer (0.05 M KH_2PO_4, 0.075 M $NaHCO_3$, 0.3 M NaCl, pH 6.80). The pH was then adjusted and maintained at 6.80 ± 0.01 for 180 min at 37 °C.

2.14.3. Sampling and digestion termination

- Samples (0.5 mL) were collected at predefined time points:
- Gastric phase: 0, 5, 10, 15, 30 min (G0–G30)
- Intestinal phase: 0, 5, 10, 15, 30, 60, 120, 180 min (I0–I180)

Immediately after collection, each aliquot was mixed with 3 mL of 90 % (*v*/v) ethanol to terminate enzyme activity. The ethanol-preserved supernatants were stored at −40 °C until further analysis.

2.14.4. Analysis

Prior to analysis, samples were thawed and diluted with deionized water as required to ensure analyte concentrations fell within the optimal assay range. All measurements were performed in triplicate, and results were expressed as relative standard deviation (RSD). Standard calibration curves were prepared for each assay, confirming good linearity (R^2 values >0.99). Sample dilutions were adjusted to maintain final analyte concentrations within the validated linear range.

2.15. Statistical analysis

All data were analyzed using two-factor Analysis of Variance (ANOVA) to assess the effects of pressure (300, 450, and 600 MPa) and time (5 and 10 min) as fixed factors on various dependent variables. Specifically, a two-way ANOVA was used to evaluate the main effects of pressure and time, as well as their interaction. Following the ANOVA, Duncan's multiple range test was applied to determine significant differences between treatment means.

In some cases, Multivariate Analysis of Variance (MANOVA) was also employed to assess the overall effect of the combined treatment conditions (pressure × time) on multiple dependent variables simultaneously. Univariate ANOVAs were conducted for each dependent variable to provide more detailed insights into the specific effects of each treatment factor.

Statistical analyses were performed using IBM SPSS Statistics 26, and data are presented as means ± standard deviation (SD). All experiments were conducted in triplicate to ensure reliability and reproducibility of results.

3. Results and discussion

3.1. Changes in pH, TSS, AA, and turbidity of green tea infusions after HPP

3.1.1. pH

Table 1 presents the changes in pH, TSS, AA, and turbidity of green tea infusions after HPP. The pH values of the samples ranged from a minimum of 5.44 (CB + Ps and HB + Ps) to a maximum of 5.52 (CB450:10 and CB600:5), indicating that all samples were slightly acidic. While the pH values of the HB + Ps and CB + Ps samples were similar, the data suggest that pasteurization (Ps) reduces the pH of the tea infusion. The reduction is likely due to the higher extraction of phenolic compounds and organic acids during pasteurization.

On the other hand, HPP induced an increase in pH, but this change was only statistically significant ($p < 0.05$) when the treatment duration was extended to 10 min. Specifically, increasing the holding time from 5 to 10 min led to a pH increase at both 300 MPa (from 5.47 to 5.51) and 450 MPa (from 5.48 to 5.52). At 600 MPa, however, the pH values remained statistically unchanged ($p > 0.05$).

3.1.2. Total soluble solids (TSS)

Total soluble solids (TSS) in green tea, comprising phenolic compounds, carbohydrates, alkaloids, pigments, amino acids, and vitamins, serve as a key indicator of overall flavor and infusion quality. Soluble carbohydrates contribute sweetness, while amino acids add brothy and umami notes to the tea infusion.

The TSS content of HB + Ps was slightly higher (0.63 g/100 g) compared to CB + Ps (0.60 g/100 g), although this difference was statistically insignificant ($p > 0.05$). This minor increase in TSS for the hot-brewed samples is expected, as higher steeping temperatures facilitate the disruption of cell walls, allowing for greater phenolic compound extraction.

Moderate HPP pressure (300 and 450 MPa) further enhanced the extraction of TSS from the cold-brewed (CB) samples. The TSS content increased from 0.60 g/100 g in CB + Ps to a range of 0.61 g/100 g at 300 MPa and 0.64 g/100 g at 450 MPa. This result aligns with findings from Chen et al. (2009), who reported that ultra-high pressure processing (UHPP) at 100, 300, and 500 MPa improved the extraction of free amino acids and polyphenols, though water-soluble saccharides were extracted in lower amounts. The increase in TSS at these pressures can be attributed to elevated pressure, which increases the solubility of bioactive compounds and alters cell structure, facilitating solvent penetration and the release of intracellular contents.

However, a significant reduction in TSS content ($p < 0.05$) was observed when the pressure was increased from 450 MPa to 600 MPa. At 600 MPa, TSS values were the lowest among all samples, with CB600:5 showing 0.57 g/100 g and CB600:10 showing 0.59 g/100 g. The reduction in TSS content at higher pressures is consistent with previous studies, which suggest that pressures exceeding 400–450 MPa can negatively impact protein composition (Tepsongkroh, Thaihuttakij, Supawong, & Jangchud, 2023) and lead to degradation of certain polyphenols (Jeż, Wiczkowski, Zielińska, Białobrzewski, & Błaszczak, 2018).

3.1.3. %transmittance

The %transmittance of the tea samples ranged from a minimum of 94.53 % (in CB450:10) to a maximum of 96.47 % (in CB600:5). Cold-brewed (CB) tea had a higher transmittance and a lower turbidity than hot-brewed (HB) tea. This difference is because cold water extracts fewer compounds, such as polyphenols, proteins, and caffeine, compared to hot water. These compounds typically interact to form complexes known as “tea cream,” which precipitate upon cooling and contribute to the turbidity of the tea. Turbidity in tea is associated with increased bitterness and reduced enjoyment of astringency, brightness, clarity, and overall taste. Therefore, cold-brewed tea may be less bitter

Table 1
Physicochemical properties of cold-brewed green tea under different treatments: Cold Brew (CB), Cold Brew with HPP (CB300:5, CB300:10, CB450:5, CB450:10, CB600:5, CB600:10), Cold Brew and Hot Brew with pasteurization at 65 °C for 30 min (CB + Ps and HB + Ps).

Treatment	TSS (g/100 g)	pH	Ascorbic acid (mg/mL × 10^{-2})	Chlorophyll *a* (mg/mL × 10^{-2})	Chlorophyll *b* (mg/mL × 10^{-2})	Total chlorophyll (mg/mL × 10^{-2})	Color				Transmittance (%)
							L*	a*	b*	ΔE	
CB	0.60 ± 0.00^{abc}	5.45 ± 0.02^{abc}	**1.87 ± 0.41^{f}**	**0.0067 ± 0.0015^{c}**	**0.0089 ± 0.0022^{d}**	0.0158 ± 0.0028^{d}	3.26 ± 0.03^{d}	−1.01 ± 0.01^{d}	2.99 ± 0.02^{d}	–	95.72 ± 1.32^{ab}
CB300:5	0.61 ± 0.03^{abc}	5.47 ± 0.01^{abc}	**1.17 ± 0.23^{g}**	**0.0064 ± 0.0014^{c}**	**0.0123 ± 0.0021^{c}**	0.0186 ± 0.0031^{c}	3.27 ± 0.04^{d}	−1.02 ± 0.09^{d}	2.98 ± 0.14^{d}	0.11 ± 0.06^{e}	96.02 ± 1.10^{a}
CB300:10	0.63 ± 0.05^{ab}	5.51 + 0.03^{abc}	**2.48 ± 0.29^{de}**	**0.0092 ± 0.0010^{b}**	**0.0166 ± 0.0023^{b}**	0.0257 ± 0.0030^{b}	2.72 ± 0.07^{f}	−0.80 ± 0.08^{abc}	2.64 ± 0.05^{e}	0.68 ± 0.09^{c}	95.74 ± 1.13^{ab}
CB450:5	0.64 ± 0.05^{a}	5.48 ± 0.02^{abc}	**3.69 ± 0.15^{b}**	**0.0064 ± 0.0010^{c}**	**0.0118 ± 0.0018^{c}**	0.0183 ± 0.0015^{c}	3.03 ± 0.02^{e}	−0.90 ± 0.08^{cd}	2.92 ± 0.10^{d}	0.28 ± 0.07^{d}	96.46 ± 1.15^{a}
CB450:10	0.64 ± 0.05^{a}	5.52 ± 0.07^{a}	**4.37 ± 0.39^{a}**	**0.0158 ± 0.0026^{a}**	**0.0203 ± 0.0024^{a}**	0.0362 ± 0.0026^{a}	3.87 ± 0.09^{c}	−1.30 ± 0.08^{e}	3.62 ± 0.03^{c}	0.93 ± 0.12^{b}	94.53 ± 0.59^{b}
CB600:5	0.57 ± 0.05^{c}	5.52 ± 0.06^{a}	**2.88 ± 0.58^{cd}**	**0.0062 ± 0.0020^{c}**	**0.0096 ± 0.0024^{d}**	0.0158 ± 0.0021^{d}	3.24 ± 0.04^{d}	−0.89 ± 0.04^{bcd}	2.98 ± 0.06^{d}	0.14 ± 0.04^{e}	96.47 ± 0.16^{a}
CB600:10	0.59 ± 0.08^{bc}	5.51 ± 0.03^{ab}	**3.08 ± 0.68^{c}**	**0.0091 ± 0.0007^{b}**	**0.0164 ± 0.0014^{b}**	0.0250 ± 0.0019^{b}	4.30 ± 0.07^{a}	−1.35 ± 0.09^{e}	3.85 ± 0.04^{b}	1.39 ± 0.12^{a}	94.63 ± 0.65^{b}
CB + Ps	0.60 ± 0.00^{abc}	5.44 ± 0.02^{c}	**2.34 ± 0.34^{ef}**	**0.0065 ± 0.0018^{c}**	**0.0133 ± 0.0018^{c}**	0.0194 ± 0.0018^{c}	3.10 ± 0.03^{e}	−0.77 ± 0.05^{ab}	2.97 ± 0.10^{d}	0.30 ± 0.02^{d}	95.54 ± 1.43^{ab}
HB + Ps	0.63 ± 0.05^{ab}	5.44 ± 0.02^{bc}	**2.13 ± 0.47^{ef}**	**0.0080 ± 0.0007^{b}**	**0.0120 ± 0.0011^{c}**	0.0202 ± 0.0013^{c}	4.09 ± 0.06^{b}	−0.70 ± 0.07^{a}	4.08 ± 0.11^{a}	1.41 ± 0.07^{a}	94.78 ± 0.52^{b}

Values expressed mean ± SD (**$n = 3$**). Data of different alphabets were different with statistical significance ($p < 0.05$). Lightness (**L***), redness (**a***), yellowness (**b***), Total color difference (**ΔE**), Total soluble solids (**TSS**).

and astringent than hot-brewed tea.

Although %transmittance increased slightly with higher pressure (from 96.02 % at 300 MPa to 96.47 % at 600 MPa), these differences were not statistically significant ($p > 0.05$). This suggests that while higher pressure led to a slight improvement in clarity, the change was not sufficient to reach statistical significance.

However, HPP duration had a notable effect on the clarity and optical properties of the tea infusion. Increasing the holding time from 5 to 10 min led to a decrease in %transmittance in the CB tea samples. Specifically, the %transmittance decreased from 95.74 % to 94.53 % at 450 MPa and from 95.74 % to 94.63 % at 600 MPa. The reduction in % transmittance observed at longer holding times is attributed to enhanced extraction of chemical constituents, leading to a cloudier tea with less light passing through the infusion.

3.1.4. Ascorbic acid (AA) content

HPP is widely recognized for enhancing the extraction of bioactive compounds from plants and fruits; however, its effect on AA content is highly variable. This variability is primarily influenced by key processing parameters, such as pressure, duration, temperature, and the food matrix.

Table 1 shows the AA content in tea infusions across different HPP treatments, compared to hot brewing (HB) and pasteurization (Ps). The lowest AA contents were observed in the cold-brewed tea (CB) sample (1.87×10^{-2} mg/mL) and the CB300:5 sample (1.17×10^{-2} mg/mL). Despite the similar AA levels between cold-brewed tea (CB + Ps) and hot-brewed tea (HB + Ps), the difference was not statistically significant ($p > 0.05$). Specifically, the AA contents for CB + Ps and HB + Ps were 2.34×10^{-2} mg/mL and 2.13×10^{-2} mg/mL, respectively.

Although AA content increased with rising HPP pressure, the rate of increase diminished as pressure levels increased. This observation is consistent with the findings of Tewari, Sehrawat, Nema, and Kaur (2017), which indicated that lower pressure, lower temperature, and shorter processing times generally help maintain or even boost AA content. Similar effects have been noted for other bioactive compounds, such as carotenoids, anthocyanins, and phenolics (Tewari et al., 2017). HPP caused cellular rupture, releasing cytosolic contents into the extracellular space, which subsequently increased AA levels.

All HPP-treated samples demonstrated superior AA retention compared to conventional pasteurization, except for the CB300:5 sample, which had the lowest AA content among the HPP-treated samples. This highlights the benefit of using HPP to preserve or enhance nutrient levels. However, it is critical to optimize processing conditions—particularly minimizing pressure duration and avoiding excessively high-pressure levels—to maximize nutrient retention and extraction efficiency.

3.2. Color measurement

Table 1 highlights the significant effects ($p < 0.05$) of different treatments on the color of the cold-brewed green tea infusions. The L (lightness)* values indicated that CB300:10 infusions were the darkest (2.72), while CB600:10 infusions were the lightest (4.30). The infusion was lighter at higher pressures (450 and 600 MPa) compared to the 300 MPa treatment, and this lightening effect was further intensified by longer HPP durations. This suggests that prolonged HPP treatment promotes the degradation of green tea pigments, resulting in an increase in the L* value (lighter appearance) due to the breakdown of certain chromophores.

The lighter appearance of hot-brewed (HB) tea compared to cold-brewed (CB) tea in our study aligns with the findings of Chiang et al. (2020), who observed that increasing cold-brewing time resulted in darker tea (lower L* values) and more yellow tones (b values). However, no significant difference in L and b* values was found between CB and HB infusions after 14 h of cold brewing. In contrast, Lin et al. (2014) reported that cold-brewed tea yielded a lighter color than hot-brewed tea, which contrasts with our results. This discrepancy may be due to differences in soaking times; Lin et al. (2014) used 24-h cold brew and a 20-min hot brew, while our study used a 12-h cold brew and 5-min hot brew durations.

Our results indicated that green tea samples with higher chlorophyll content showed increased greenness, corresponding to more negative a* values. When the HPP duration was increased from 5 to 10 min at 300 MPa, the infusion became redder, with the a* value rising from −1.02 (CB300:5) to −0.08 (CB300:10). However, this trend was reversed at 450 and 600 MPa. At 300 MPa, a 10-min HPP duration enhanced the redness of the infusion. The HB + Ps infusion exhibited the highest redness and yellowness values among all the tea infusions.

The total color difference (ΔE) of the tea infusions was calculated using untreated CB as a control. The CB300:5 infusions successfully maintained the original hue and color of the untreated CB infusions (0.11). The greatest color changes were observed in HB + Ps (1.41) and CB600:10 (1.39) infusions. Minimal ΔE values were observed in HPP samples treated for 5 min at all pressures (CB300:5 = 0.11, CB450:5 = 0.28, and CB600:5 = 0.14). Increasing the HPP duration from 5 to 10 min caused a significant color change in the infusion, with ΔE values rising to 0.68, 0.93, and 1.39 ($p < 0.05$) for CB300:10, CB450:10, and CB600:10, respectively. This substantial color change is attributed to the enhanced degradation of the tea's natural pigments and colored compounds over longer processing durations.

3.3. Total chlorophyll content

Table 1 presents the contents of chlorophyll *a*, chlorophyll *b*, and total chlorophyll in the tea infusions. The green color of tea is primarily attributed to chlorophyll *a* (blue-green) and chlorophyll *b* (yellow-green) (Yu et al., 2019). Chlorophylls are released from the delicate tea leaves during infusion and may exist as an emulsion in the tea infusion (Wang, Park, Chung, Baik, & Park, 2004).

Among the HPP treatments, CB450:10 exhibited the highest levels of chlorophyll *a* (0.0158×10^{-2} mg/mL), chlorophyll *b* (0.0203×10^{-2} mg/mL), and total chlorophyll (0.0362×10^{-2} mg/mL). At 5 min of HPP, no significant changes in chlorophyll *a* content were observed across all pressures (ranging from 0.0062 to 0.0064×10^{-2} mg/mL). However, after 10 min of HPP treatment, a significant increase in chlorophyll *a* content was observed, ranging from 0.0092×10^{-2} mg/mL to 0.0158×10^{-2} mg/mL ($p < 0.05$).

Both pasteurization and HPP treatments significantly increased the chlorophyll *b* content ($p < 0.05$). The greater stability of chlorophyll *b* compared to chlorophyll *a* explains this increase. Chlorophyll b has a 50 % lower degradation rate constant, indicating that it degrades more slowly and thus better preserves the green color (Wang et al., 2013). The strong effects of pasteurization and HPP on chlorophyll *b* led to a corresponding increase in total chlorophyll content in the tea infusions. These findings align with those of Ali, Popović, Koutchma, Warriner, and Zhu (2020), who suggested that such treatments promote the release of chlorophyll from plant cells.

Interestingly, CB600:5 showed no significant difference in total chlorophyll content (0.158×10^{-2} mg/mL) compared to the untreated CB ($p > 0.05$), suggesting that high pressure applied over a short duration (5 min) has minimal effect on chlorophyll content. However, extending the HPP duration or applying lower pressure for longer durations resulted in more substantial positive effects on chlorophyll content.

Furthermore, the relationship between chlorophyll content and the a* value changed following HPP treatment. Consistent with the findings of Lu et al. (2009), changes in chlorophyll *a* and b were negatively correlated with the a* value of the green tea infusion (*i.e.*, as chlorophyll content increased, the redness of the infusion decreased). This relationship further supports the notion that chlorophyll content is a key contributor to the green color and overall appearance of the tea infusion.

Overall, the results demonstrated that HPP is effective in sustaining

and enhancing chlorophyll content during processing, particularly with longer treatment times or lower pressures over extended durations.

3.4. Catechin and caffeine profiles

Table 2 presents a summary of the impact of HPP and thermal treatments on various catechins (catechin (C), catechin gallate (CG), gallocatechin (GC), epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), and epicatechin gallate (ECG)), as well as total catechins (TC) and caffeine levels. EGCG, EGC, ECG, and EC are typically present in fresh tea leaves in descending order of concentration (Yu et al., 2019). During infusion, chlorophyll and catechins are released from the fragile tea leaves and may exist as an emulsion in the tea (Wang et al., 2004).

Total Catechin Content: CB300:5 had the highest total catechin content (0.89 mg/mL), whereas CB600:10 had the lowest (30.90 mg/mL). The two primary contributors to total catechin content were EGC, which ranged from 0.31 to 0.53 mg/mL, and EGCG, which ranged from 0.07 to 0.22 mg/mL. The absence of C and CG in the green tea infusion may be attributed to specific processing conditions, as noted by Wong et al. (2022).

Changes in catechin content can be classified into two categories. Non-galloylated catechins (GC, EGC, and EC) exhibited higher concentrations under HPP conditions, demonstrating that cold-brewed tea (CB) preserves these compounds more effectively than hot-brewed tea (HB). In contrast, esterified catechins (EGCG, GCG, and ECG) were found in greater amounts in HB + Ps treatments, suggesting that heat treatment might have caused epimerization or the release of these catechins. Research indicates that at temperatures below 50 °C, EGCG degradation occurs *via* oxidation, while higher temperatures favor epimerization (Zeng et al., 2018). HPP may induce EGCG degradation by creating a negative activation volume and altering pH under pressure; combined with matrix effects, these factors could contribute to the observed decrease in EGCG levels. The resulting oxidation of EGCG may generate other compounds and release additional substances from the tea, which might be associated with the observed increase in TSS. The exact mechanism, however, requires further verification through product identification techniques such as LC-MS/MS or high-resolution spectroscopy. This will allow a more precise understanding of the underlying mechanisms and their contribution to changes in TSS and catechin profiles.

Catechin levels, particularly the gallated catechins (EGCG, ECG, GCG, and CG), significantly influence green tea's flavor and astringency (Wong et al., 2022). Conversely, higher molar concentrations of EGC and EC increase the sweet notes of the tea (Zhang, Cao, Granato, Xu, & Ho, 2020). Therefore, cold-brewed green tea treated with HPP is likely to produce a less astringent and bitter infusion, potentially enhancing the sweet aftertaste compared to conventional hot-brewed green tea.

Effects of HPP duration: The duration of HPP significantly influenced catechin content, with prolonged HPP (from 5 to 10 min) generally leading to a decrease in both cis- (EGC, EC, and EGCG) and trans-configured catechins (GC). Interestingly, no clear inverse or direct relationship between the concentrations of GC, EGC, EC, and C was observed, suggesting that *epi*merization of catechins was not the primary reaction during processing. Instead, catechin degradation, likely due to pressure, resulted in a decrease in total catechin content. Li, Taylor, Ferruzzi, and Mauer (2012) found that cis-configured catechins break down over time when subjected to temperatures of 120 °C, while trans-configured catechins initially increase, stabilize after 90 min, and then decrease with prolonged heating. In our study, however, changes in catechin concentrations during treatments were complex, likely involving multiple reactions such as epimerization, oxidation, and polymerization. GCG and ECG remained relatively stable under varying HPP pressures and durations.

Catechin transformations during tea processing likely involve a combination of epimerization, oxidation, and polymerization. Epimerization converts "epi-" catechins to their non-epi isomers (*e.g.*, EGCG → GCG, EGC/EC → GC/EC) and is particularly favored under elevated temperatures, following first-order kinetics (Zeng et al., 2018). Oxidation and general degradation may occur under heat, prolonged exposure, or in the presence of oxygen, metal ions, or elevated pH, producing semiquinone intermediates and leading to breakdown of susceptible catechins such as those with pyrogallol rings (Li et al., 2012). Polymerization or condensation reactions can generate oligomers (*e.g.*, dehydrodicatechins) even under low-oxygen conditions, which are often undetectable by standard HPLC focused on monomers and may contribute to changes in TSS or color (Li et al., 2012). Collectively, these mechanisms likely act concurrently, with their relative contributions depending on processing conditions.

Because each of these mechanisms, namely epimerization, oxidation/degradation, and polymerization/oligomerization, can occur under our processing conditions (heat, HPP, pH, oxygen, matrix environment), we now interpret our data as the result of a combination of these pathways, rather than attributing changes solely to one. We emphasize that the relative contribution of each pathway likely varies with treatment conditions (temperature, pressure, pH, presence of oxygen or metal ions, processing time, *etc.*), and we highlight the need for further targeted studies (*e.g.*, LC-MS/MS profiling to detect epimers and oligomers, quantification of oxidation products) to clarify their respective roles.

Caffeine Content: HB + Ps and CB300:5 showed the highest caffeine content, with values of 0.25 and 0.24 mg/mL, respectively. No significant changes in caffeine content were observed following HPP treatments, except for the CB300:5 sample ($p < 0.05$). Research by Lin,

Table 2
Average of individual catechins of cold-brewed green tea under different treatments: Cold Brew (CB), Cold Brew with HPP (CB300:5, CB300:10, CB450:5, CB450:10, CB600:5, CB600:10), Cold Brew and Hot Brew with Pasteurization at 65 °C for 30 min (CB + Ps and HB + Ps).

Tea samples	Type of catechins (mg/mL tea infusion)									
	C	CG	GC	EGC	EC	EGCG	GCG	ECG	TC	*CF*
CB	ND	ND	0.05 ± 0.01^{bc}	0.51 ± 0.04^{a}	0.08 ± 0.01^{bc}	0.12 ± 0.01^{b}	0.01 ± 0.00^{a}	0.04 ± 0.00^{b}	0.82 ± 0.04^{b}	0.19 ± 0.01^{b}
CB300:5	ND	ND	0.07 ± 0.00^{a}	0.53 ± 0.01^{a}	0.11 ± 0.00^{a}	0.12 ± 0.02^{b}	0.01 ± 0.00^{a}	0.04 ± 0.00^{b}	0.89 ± 0.02^{a}	0.24 ± 0.02^{a}
CB300:10	ND	ND	0.04 ± 0.01^{d}	0.42 ± 0.02^{c}	0.08 ± 0.01^{bc}	0.07 ± 0.01^{c}	0.01 ± 0.00^{a}	0.04 ± 0.01^{b}	0.65 ± 0.03^{ef}	0.19 ± 0.01^{b}
CB450:5	ND	ND	0.05 ± 0.01^{bcd}	0.43 ± 0.01^{bc}	0.07 ± 0.01^{cd}	0.11 ± 0.01^{b}	0.01 ± 0.00^{a}	0.04 ± 0.00^{b}	0.70 ± 0.02^{cd}	0.19 ± 0.02^{b}
CB450:10	ND	ND	0.04 ± 0.00^{cd}	0.42 ± 0.02^{c}	0.07 ± 0.01^{cd}	0.08 ± 0.01^{c}	0.01 ± 0.00^{a}	0.04 ± 0.00^{b}	0.66 ± 0.03^{de}	0.18 ± 0.01^{b}
CB600:5	ND	ND	0.04 ± 0.01^{bcd}	0.46 ± 0.01^{b}	0.09 ± 0.00^{b}	0.12 ± 0.02^{b}	0.01 ± 0.00^{a}	0.04 ± 0.00^{b}	0.73 ± 0.02^{c}	0.20 ± 0.01^{b}
CB600:10	ND	ND	0.03 ± 0.02^{d}	0.36 ± 0.02^{d}	0.07 ± 0.01^{de}	0.11 ± 0.01^{b}	0.01 ± 0.00^{a}	0.04 ± 0.01^{b}	0.61 ± 0.02^{f}	0.20 ± 0.02^{b}
CB + Ps	ND	ND	0.06 ± 0.01^{b}	0.42 ± 0.03^{c}	0.09 ± 0.01^{b}	0.11 ± 0.01^{b}	0.01 ± 0.00^{a}	0.04 ± 0.00^{b}	0.73 ± 0.02^{c}	0.21 ± 0.02^{b}
HB + Ps	ND	ND	0.02 ± 0.01^{e}	0.31 ± 0.02^{e}	0.06 ± 0.01^{e}	0.22 ± 0.01^{a}	0.01 ± 0.00^{a}	0.05 ± 0.01^{a}	0.67 ± 0.03^{de}	0.25 ± 0.02^{a}

Values expressed mean ± SD ($n = 3$). Data of different alphabets were different with statistical significance ($p < 0.05$). (+)-Catechin (C), (−)-Catechin gallate (CG), (−)-Gallocatechin (GC), (−)-Epigallocatechin (EGC), (−)-Epicatechin (EC), (−)-Epigallocatechin gallate (EGCG), (−)-Gallocatechin gallate (GCG), (−)-Epicatechin gallate (ECG), Total catechin (TC), Caffeine (*CF*), "ND" indicates values below the limit of detection. Total catechin concentration was defined as the sum of EGCG, EGC, ECG, and EC values.

Huang, and Wang (2022) showed that high-pressure processing (200–600 MPa) for short durations (1–5 min) can enhance caffeine extraction, especially when combined with higher temperatures (50 °C). Conversely, Bellumori et al. (2021) reported no significant impacts ($p > 0.05$) of HPP on caffeine content. These findings suggest that the overall effect of HPP on caffeine content is influenced by the pressure, duration of treatment, and operating temperature.

3.5. Total phenolic content (TPC) and antioxidant activity

Table 5 presents the total phenolic content (TPC) and antioxidant activities of green tea infusions, evaluated using two assays: the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability assay and the ferric reducing power (FRAP) assay. The highest TPC was observed in the HB + Ps treatment (1.70 mg GAE/mL), followed closely by CB + Ps (1.60 mg GAE/mL), indicating that heating processes are more effective for phenolic extraction than cold brewing.

TPC and Pressure Effects: Our results indicate that both pressure and time significantly enhanced the release of phenolic compounds into the tea infusion ($p < 0.05$). The highest increase in TPC after HPP was found in CB300:10, with a TPC of 1.53 mg GAE/mL, reflecting an approximate 4 % increase compared to the untreated CB (1.47 mg GAE/mL). In contrast, increasing the pressure to 600 MPa and extending the duration to 10 min resulted in a decrease in TPC levels, with CB600:10 exhibiting the lowest TPC value among all treatments (1.40 mg GAE/mL). This phenomenon may be attributed to the modification of hydrogen bonds in phenolic compounds under high pressure, leading to degradation and subsequent reduction in TPC (Akhmazillah, Farid, & Silva, 2013).

Comparison with Previous Studies: Previous studies focusing on HPP during tea extraction have reported varying effects on phenolic compound retention. For instance, Uzuner and Evrendilek (2019) observed higher TPC and antioxidant activity at 300 MPa compared to 500 MPa during tea brewing, while Šeremet et al. (2021) found elevated levels of TPC, EGCG, and ECG at 200 MPa compared to 500 MPa. These studies, however, focused on HPP during the preparation phase, rather than as a post-processing treatment, meaning their optimal conditions may differ. These authors suggest that optimal HPP conditions vary significantly depending on the specific tea type.

Antioxidant Activity and TPC: Both DPPH radical scavenging activity and the reducing power of green tea infusions showed a positive relationship with TPC. This correlation confirms that phenolic compounds were primarily responsible for the antioxidant activities of green tea, specifically in terms of reducing power and free radical scavenging capacity. HB + Ps treatment yielded the highest antioxidant activity, with DPPH and FRAP values of 14.13 mM TE/mL and 58.13 mM $FeSO_4$/mL, respectively, aligning with its high TPC content.

HPP treatments significantly increased TPC, DPPH radical scavenging ability, and FRAP values ($p < 0.05$). Antioxidant activities for HPP-treated CB ranged from 10.36 mM TE/mL (CB600:10) to 12.65 mM TE/mL (CB300:10) for DPPH and from 48.56 mM $FeSO_4$/mL (CB600:10) to 51.85 mM $FeSO_4$/mL (CB300:5) for FRAP. Among all HPP conditions, 300 MPa treatments were the most effective in enhancing both TPC and antioxidant activity. The DPPH scavenging ability increased significantly at 300 MPa but declined at higher pressures (450 MPa and 600 MPa). While extending the duration from 5 to 10 min generally improved the antioxidant effects at lower pressures, increasing the duration at 600 MPa reduced antioxidant activity. This reduction can likely be attributed to the stability of phenolic compounds in tea.

The increase in TPC after HPP is in line with what has been found in other plant-based foods, like carrots, spinach, strawberries, blackberry purées, and bee pollen drinks. The mechanical disruption of cell walls, induced by either high heat or high pressure, facilitates the release of phenolic compounds. This mechanism accounts for the notable increase in TPC levels observed after heating (9–15 %) and HPP (0.8–4 %). For example, microscopy analysis revealed small pieces of cell debris in CB infusions at 10× magnification (Fig. 1a) and flattened cells in CB600:5

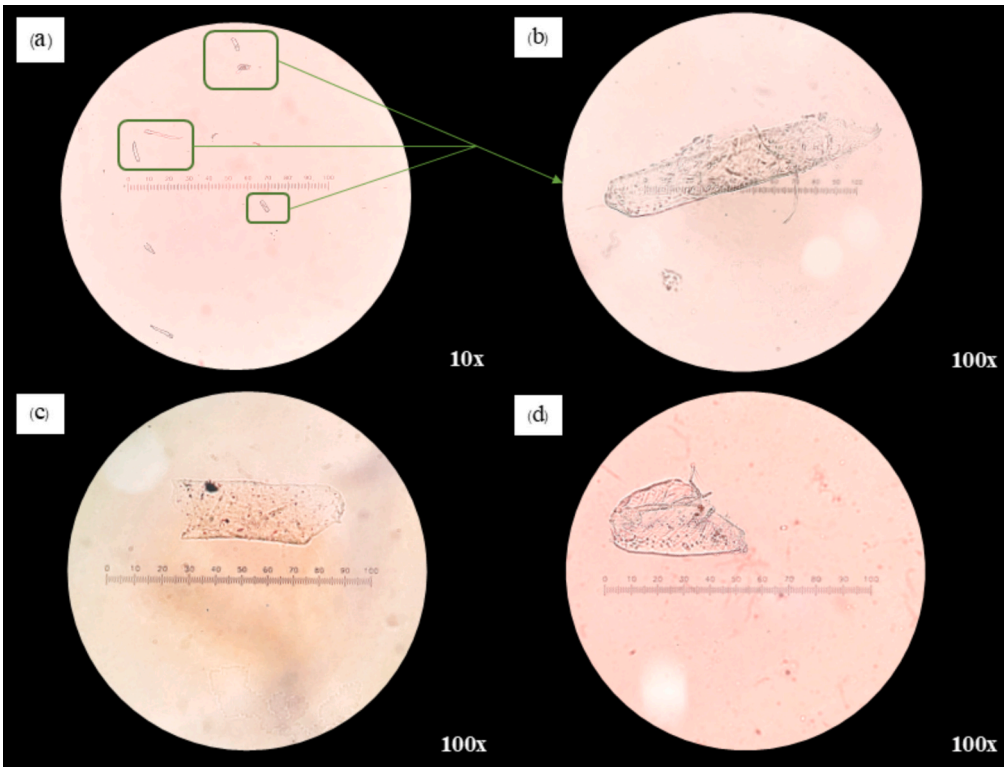


Fig. 1. Microscopic images of plant cell debris in green tea infusions. (a) Control (CB) under 10× magnification; (b) Control (CB) under 100× magnification; (c) CB600:5 (HPP 600 MPa for 5 min) under 100× magnification; (d) CB600:10 (HPP 600 MPa for 10 min) under 100× magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and CB600:10 infusions at 100× magnification (Fig. 1c and d).

Phenolic compounds contribute to the bitter and astringent notes in tea (Meyer, White, McCormack, & Niemeyer, 2023). Therefore, it can be inferred that green tea processed through hot brewing or pasteurization might possess a more pronounced bitter taste when compared to cold-brewed green tea treated with HPP.

Antioxidant Strength of Catechins: The antioxidant strength of individual catechins is dictated by their molecular structure, particularly the number and position of hydroxyl groups (OH) (Sheng et al., 2023). EGCG, which possesses the highest number of phenolic hydroxyl groups, exhibits the strongest antioxidant activity (Gan, Li, Sui, & Corke, 2018). The maximal EGCG content found in HB + Ps (Table 3) likely contributes significantly to the elevated antioxidant activities. Conversely, EGCG degradation following HPP treatment may be responsible for the reduction in overall antioxidant capacity. Catechins with a galloyl substituent, such as EGCG and ECG, demonstrate superior free radical scavenging activity compared to their non-gallated counterparts (EGC and EC).

However, while catechins are prominent contributors to antioxidant activity, they may not entirely account for the measured TPC values or antioxidant activity (Meyer et al., 2023). The highest total catechin levels in CB and CB300:5 did not correlate with the highest TPC and antioxidant activity (Table 3). This suggests that other phenolic compounds in green tea, such as flavonols and phenolic acids, also play significant roles in its overall antioxidant capacity.

3.6. Amino acid profile

Table 4 summarizes the effects of high-pressure processing (HPP) on the amino acid profile of green tea infusions. Previous studies indicate that amino acids contribute approximately 70 % of the umami taste intensity in green tea, with glutamic acid and glutamine being the primary contributors (Chen et al., 2022), and aspartic acid playing a significant supporting role (Ruan et al., 2019). Theanine, a key amino acid in tea, was not quantified in this study due to limitations in analytical standards and methods. However, Liu, Ueno, Shimada, and Araki (2019) observed that HPP applied at pressures of 300, 500, and 700 MPa for extended durations (10, 30, and 60 min) increased the formation of free amino acids in green tea leaves, likely through protein hydrolysis. Specifically, the production of theanine might have been enhanced due to the increased availability of its precursors (glutamic acid and ethylamine) for the enzyme theanine synthase. The decrease in glutamic acid observed in this study suggests a potential increase in theanine synthesis. However, these findings were based on HPP as an alternative to the steaming process, and further investigation is required to explore the specific effects of HPP as a post-processing treatment for cold-brewed green tea. Future studies should directly measure theanine levels to better understand how HPP affects the amino acid content and flavor profile of cold-brewed green tea.

Amino Acid Composition: In this study, the major amino acids—aspartic acid, glutamic acid, and serine—constituted over 90 % of the total amino acids (TAA) quantified in the green tea samples. Aspartic acid levels ranged from 16.17 mg/mL, glutamic acid from 3.21 to 4.41 mg/mL, and serine from 0.83 to 2.19 mg/mL. The CB300:10 infusion exhibited the highest glutamic acid content (4.41 mg/mL), reinforcing its potential for an intense umami flavor. The glutamine content was also notable, especially in CB300:5 and CB300:10, both showing 0.02 mg/mL.

Conversely, serine content was highest in the untreated CB and several HPP treatments, including CB450:5 (2.19 mg/mL), CB (1.98 mg/mL), and CB600:5 (1.96 mg/mL). Serine and proline are known to contribute to the sweetness of green tea (Ye et al., 2022), enhancing its overall flavor profile. Proline was undetectable in CB300:10 and CB450:5 samples, suggesting that certain HPP treatments can impact amino acid composition, especially those contributing to sweetness. Other sweet-tasting amino acids, including L-alanine, L-glycine, L-serine, L-proline, and L-threonine (Zhang et al., 2020), were also identified in the samples.

Arginine levels were higher in HPP-treated samples compared to the controls (CB and HB), with significant increases noted in the CB300:10 and CB450:10 treatments. Glutamine, glutamic acid, and arginine are known to accumulate as primary metabolites, contributing to both umami and sweet flavors (Zhang, Huang, et al., 2024), suggesting that enhanced arginine levels may have played a significant role in the flavor profile of the HPP-treated green tea.

Amino Acid Variability: However, several amino acids, including asparagine, histidine, and tryptophan, were undetectable in all samples. Additionally, threonine, another amino acid contributing to the sweetness of green tea, was absent in CB600:10 but present in other treatments, with values ranging from 0.28 mg/mL (CB + Ps) to 0.34 mg/mL (CB300:5). These findings suggest that HPP treatment at 600 MPa for 10 min (CB600:10) might have a negative impact on the sweetness of the tea infusion.

Total Amino Acid Content (TAA): Among the treatments, HB + Ps exhibited the highest total amino acid content (TAA) at 26.25 mg/mL, while CB600:10 showed the lowest TAA (22.5 mg/mL). This suggests that traditional high-temperature brewing methods may be more effective in maximizing amino acid content compared to certain HPP conditions. In comparison to untreated CB (23.61 mg/mL), HPP treatments generally resulted in a slight increase in TAA, with values ranging from 22.50 to 25.71 mg/mL. The increase in TAA is likely due to HPP-induced disruption of cellular structure and accelerated proteolysis (Liu et al., 2019). However, the lower TAA content observed in CB600:10 suggests that excessive pressure and prolonged treatment times associated with HPP may reduce the concentration of free amino acids. Zhang, Yan, et al. (2024) explained that high pressures can lead to structural modifications that cause amino acids to interact with other substances, such as polyphenols, which may reduce the free amino acid pool.

Effect of HPP Duration on TAA: At 300 MPa and 450 MPa, increasing the HPP duration enhanced TAA content. Conversely, at 600 MPa, extending the treatment time from 5 to 10 min significantly lowered TAA, confirming that over-processing compromises the stability of amino acids. For example, CB600:5 had the highest total essential amino acid (TEAA) content (1.18 mg/mL), whereas CB600:10 exhibited a 55 % reduction in TEAA, yielding only 0.53 mg/mL. A similar decrease was also observed at 450 MPa. These findings highlight the detrimental impact of prolonged HPP treatment on essential amino acid stability.

Table 3
Total phenolic content (TPC), DPPH radical scavenging ability (DPPH) and ferric-reducing antioxidant power (FRAP) of cold-brewed green tea under different treatments: Cold Brew (CB), Cold Brew with HPP (CB300:5, CB300:10, CB450:5, CB450:10, CB600:5, CB600:10), Cold Brew and Hot Brew with Pasteurization at 65 °C for 30 min (CB + Ps and HB + Ps).

Tea samples	TPC (mg GAE/mL)	DPPH (mM TE/mL)	FRAP (mM FeSO4/mL)
CB	1.47 ± 0.03^{d}	12.10 ± 1.14^{b}	49.93 ± 0.66^{e}
CB300:5	1.50 ± 0.03^{cd}	12.21 ± 0.81^{b}	52.57 ± 0.98^{b}
CB300:10	1.53 ± 0.03^{c}	12.65 ± 0.70^{b}	51.85 ± 0.80^{bc}
CB450:5	1.48 ± 0.02^{d}	11.59 ± 1.25^{bc}	51.43 ± 0.56^{cd}
CB450:10	1.49 ± 0.03^{d}	11.76 ± 1.53^{b}	50.53 ± 0.60^{de}
CB600:5	1.49 ± 0.04^{d}	11.32 ± 0.45^{c}	50.08 ± 0.47^{e}
CB600:10	1.40 ± 0.02^{e}	10.36 ± 1.02^{c}	48.56 ± 0.74^{f}
CB + Ps	1.60 ± 0.03^{b}	12.26 ± 0.53^{b}	50.04 ± 1.28^{e}
HB + Ps	1.70 ± 0.02^{a}	14.13 ± 1.06^{a}	58.13 ± 0.58^{a}

Values expressed mean ± SD ($n = 3$). Data of different alphabets were different with statistical significance ($p < 0.05$).

3.7. Electronic nose (E-nose)

Principal component analysis (PCA) was performed to analyze the electronic nose (E-nose) data and explore the variations in volatile

Table 4
Average of amino acids of cold-brewed green tea under different treatments: Cold Brew (CB), Cold Brew with HPP (CB300:5, CB300:10, CB450:5, CB450:10, CB600:5, CB600:10), Cold Brew and Hot Brew with Pasteurization at 65 °C for 30 min (CB + Ps and HB + Ps).

Amino acids (mg/mL)	Tea samples								
	CB	CB300:5	CB300:10	CB450:5	CB450:10	CB600:5	CB600:10	CB + Ps	HB + Ps
Asn	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Asp	16.17 ± 0.16^{c}	17.05 ± 0.52^{bc}	16.95 ± 0.20^{bc}	16.83 ± 0.26^{bc}	17.61 ± 0.42^{b}	17.32 ± 0.20^{b}	16.20 ± 1.02^{c}	18.75 ± 0.75^{a}	19.33 ± 0.98^{a}
Ser	1.98 ± 0.08^{a}	1.06 ± 0.11^{b}	0.85 ± 0.06^{b}	2.19 ± 0.21^{a}	0.93 ± 0.05^{b}	1.96 ± 0.35^{a}	0.87 ± 0.17^{b}	1.12 ± 0.11^{b}	0.83 ± 0.15^{b}
Ala	0.41 ± 0.04^{a}	0.38 ± 0.06^{a}	0.39 ± 0.07^{a}	0.39 ± 0.08^{a}	0.38 ± 0.07^{a}	0.41 ± 0.03^{a}	0.41 ± 0.04^{a}	0.47 ± 0.03^{a}	0.43 ± 0.05^{a}
Gly	0.04 ± 0.01^{b}	0.06 ± 0.01^{a}	0.03 ± 0.01^{bcd}	0.02 ± 0.00^{cd}	0.02 ± 0.00^{cd}	0.04 ± 0.00^{b}	0.01 ± 0.01^{d}	0.03 ± 0.02^{b}c	0.03 ± 0.01^{bc}
Gln	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Thr	0.31 ± 0.03^{ab}	0.34 ± 0.02^{a}	0.32 ± 0.02^{ab}	0.31 ± 0.04^{ab}	0.30 ± 0.02^{ab}	0.32 ± 0.01^{ab}	0.00 ± 0.00^{c}	0.28 ± 0.05^{b}	0.20 ± 0.03^{ab}
Cys	0.30 ± 0.05^{a}	0.25 ± 0.03^{ab}	0.23 ± 0.06^{bc}	0.18 ± 0.06^{c}	0.24 ± 0.02^{abc}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
Glu	3.21 ± 0.28^{d}	3.47 ± 0.44^{bcd}	4.41 ± 0.40^{a}	3.48 ± 0.42^{bcd}	4.38 ± 0.67^{ab}	4.01 ± 0.74^{abcd}	3.78 ± 0.46^{abcd}	3.42 ± 0.18^{cd}	4.15 ± 0.41^{abc}
Pro	0.16 ± 0.02^{ab}	0.19 ± 0.04^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.12 ± 0.02^{b}	0.18 ± 0.04^{a}	0.13 ± 0.02^{b}	0.12 ± 0.02^{b}	0.19 ± 0.02^{a}
Lys	0.07 ± 0.01^{a}	0.07 ± 0.02^{a}	0.08 ± 0.02^{a}	0.07 ± 0.01^{a}	0.08 ± 0.02^{a}	0.08 ± 0.03^{a}	0.08 ± 0.02^{a}	0.05 ± 0.02^{a}	0.06 ± 0.04^{a}
His	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Arg	0.16 ± 0.01^{c}	0.33 ± 0.13^{abc}	0.44 ± 0.11^{ab}	0.29 ± 0.18^{bc}	0.37 ± 0.05^{ab}	0.48 ± 0.09^{a}	0.46 ± 0.10^{ab}	0.27 ± 0.07^{bc}	0.14 ± 0.03^{c}
Val	0.08 ± 0.03^{a}	0.09 ± 0.02^{a}	0.07 ± 0.04^{a}	0.08 ± 0.04^{a}	0.09 ± 0.03^{a}	0.10 ± 0.02^{a}	0.09 ± 0.04^{a}	0.09 ± 0.04^{a}	0.09 ± 0.04^{a}
Met	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Tyr	0.13 ± 0.03^{a}	0.12 ± 0.02^{a}	0.12 ± 0.01^{a}	0.12 ± 0.01^{a}	0.13 ± 0.02^{a}	0.13 ± 0.02^{a}	0.13 ± 0.02^{a}	0.13 ± 0.01^{a}	0.14 ± 0.02^{a}
Ile	0.13 ± 0.02^{a}	0.10 ± 0.03^{a}	0.11 ± 0.03^{a}	0.10 ± 0.03^{a}	0.10 ± 0.02^{a}	0.11 ± 0.03^{a}	0.09 ± 0.02^{a}	0.11 ± 0.03^{a}	0.12 ± 0.03^{a}
Leu	0.13 ± 0.02^{ab}	0.11 ± 0.03^{ab}	0.11 ± 0.03^{ab}	0.08 ± 0.03^{b}	0.11 ± 0.02^{ab}	0.11 ± 0.02^{ab}	0.10 ± 0.01^{ab}	0.14 ± 0.02^{a}	0.11 ± 0.03^{ab}
Phe	0.33 ± 0.07^{abc}	0.27 ± 0.10^{bc}	0.35 ± 0.07^{ab}	0.29 ± 0.11^{bc}	0.16 ± 0.02^{c}	0.46 ± 0.05^{a}	0.16 ± 0.16^{c}	0.33 ± 0.06^{abc}	0.32 ± 0.13^{abc}
Try	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
TAA	23.61 ± 0.23^{ef}	23.91 ± 0.38^{de}	24.50 ± 0.57^{bcde}	24.41 ± 0.47^{cde}	25.02 ± 0.38^{abcd}	25.71 ± 0.72^{ab}	22.50 ± 0.69^{f}	25.31 ± 0.79^{abc}	26.25 ± 1.30^{a}
TEAA	1.05 ± 0.08^{ab}	0.98 ± 0.15^{ab}	1.05 ± 0.08^{ab}	0.93 ± 0.10^{b}	0.84 ± 0.02^{b}	1.18 ± 0.02^{a}	0.53 ± 0.20^{c}	1.00 ± 0.07^{ab}	1.02 ± 0.14^{ab}
TNEAA	22.56 ± 0.30^{cd}	22.93 ± 0.23^{cd}	23.45 ± 0.63^{bc}	23.48 ± 0.40^{bc}	24.18 ± 0.37^{ab}	24.53 ± 0.71^{ab}	21.97 ± 0.80^{d}	24.31 ± 0.85^{ab}	25.24 ± 1.15^{a}

Values expressed mean ± SD ($n = 3$). Data of different alphabets were different with statistical significance ($p < 0.05$). L-histidine (His), taurine (Tau), L-serine (Ser), glutamine (Gln), L-arginine (Arg), glycine (Gly), aspartic acid (Asp), L-glutamic acid (Glu), L-threonine (Thr), L-alanine (Ala), proline (Pro), L-cysteine (Cys), L-lysine (Lys), L-tyrosine (Tyr), methionine (Met), L-valine (Val), L-isoleucine (Ile), L-leucine (Leu), *L*-phenylalanine (Phe). Total non-essential amino acids (TNEAA), Total essential amino acids (TEAA). TNEAA concentration was defined as the sum of EGCG, EGC, ECG, and EC values.
"ND" indicates values below the limit of detection.

profiles across the different tea samples. As shown in Fig. 2a, the first two principal components (PC1 and PC2) explained 70.90 % of the total variance, with PC1 accounting for 53.83 % and PC2 explaining 17.07 %. These principal components effectively captured the main variations in

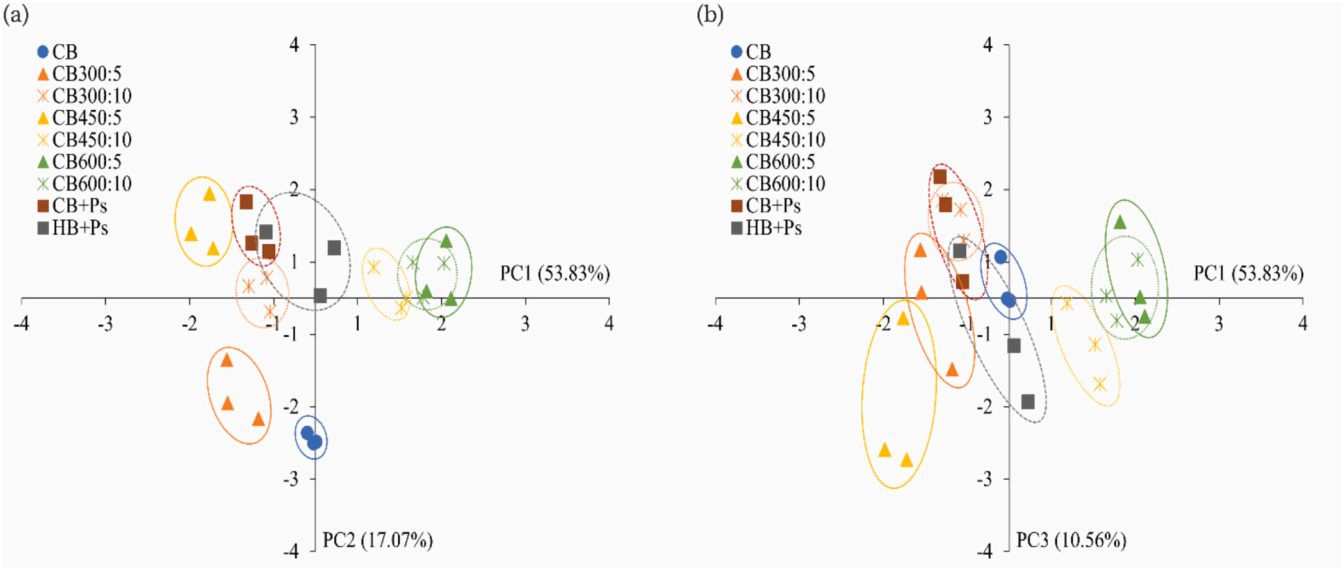


Fig. 2. PCA plot (E-nose analysis) showing volatile profiles of cold-brewed green tea under different treatments: Cold Brew (CB), Cold Brew with HPP (CB300:5, CB300:10, CB450:5, CB450:10, CB600:5, CB600:10), Cold Brew and Hot Brew with Pasteurization at 65 °C for 30 min (CB + Ps and HB + Ps). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the E-nose profiles. PC1 is primarily associated with sensor responses to compounds such as hydrogen, gaseous air contaminants, and ozone, which were detected by sensors 2, 14, 11, 10, and 1. On the other hand, PC2 appears to be more influenced by sensors 3 and 8, which are sensitive to organic solvent vapors and LPG/butane, as detailed in Table 5.

The PCA analysis revealed clear clustering patterns among the samples. The CB control was distinctly separated from the treated samples, including CB300:10, CB450:5, CB + Ps, and HB + Ps. This separation underscores the substantial impact of both HPP and pasteurization treatments on the volatile profile of the tea infusions. The CB450:10, CB600:5, and CB600:10 samples clustered closely together, indicating that a specific combination of pressure and duration led to a highly similar volatile profile. This suggests that the pressure and processing duration are key factors in shaping the volatile compounds.

The results show that increasing the pressure during HPP from 300 MPa to 600 MPa significantly altered the volatile compound profile in cold-brewed tea. Additionally, extending the HPP duration from 5 to 10 min at 300 MPa and 450 MPa induced a noticeable shift in the volatile profile. However, at 600 MPa, extending the treatment time to 10 min did not lead to further significant changes in the volatile profile. This indicates a saturation point for volatile compound changes at higher pressures and extended durations.

An intriguing observation from the PCA was that pasteurization resulted in a similar volatile profile for both hot-brewed and cold-brewed teas. This suggests that the thermal post-processing treatment may mask some of the inherent differences in volatile composition that arise from the brewing methods (cold-brew *vs.* hot-brew). Thus, pasteurization may diminish the impact of brewing methods on the volatile profile, resulting in a more uniform composition.

The PC1 *vs.* PC3 plot in Fig. 2b clearly differentiates a distinct group comprising CB450:10, CB600:5, and CB600:10, which are separated from the other samples. PC3, accounting for 10.56 % of the total variance, appears to be influenced by sensors 13 and 15, which are sensitive to LP gas and solvent vapors (Table 5). This component reveals subtle variations in the volatile profiles that seem to be associated with the combined high-intensity effects of pressure and duration. These findings suggest that PC3 captures important nuances in the volatile profile, which might be linked to the more extreme processing conditions.

Given the distinct chemical shifts observed in the PCA analysis, formal sensory evaluation is recommended for future studies to assess the impact of HPP on the final flavor characteristics of cold-brewed green tea. Formal sensory analysis would provide a more holistic understanding of how these changes in volatile compounds influence the sensory experience, particularly in terms of aroma and overall flavor quality.

Table 5
List of response of the *E*-nose sensors.

Number of sensors	Gas sensor types	Detection range (ppm)
Sensor 1	Hydrogen H_2 Gas Sensor	10–4000
Sensor 2	LPG, Propane, Hydrogen, Methane, Butane, Smoke	300–10,000
Sensor 3	Organic Solvent Vapor Sensor	50–5000
Sensor 4	Alcohol, Ethanol, Smoke	10–1000
Sensor 5	Ammonia NH_3 gas sensor	30–300
Sensor 6	Methane, Natural gas	300–10,000
Sensor 7	Ammonia	30–300
Sensor 8	LPG, Butane	200–10,000
Sensor 9	For Air Quality Various, Air Pollution	1–30
Sensor 10	Hydrogen	100–10,000
Sensor 11	Air Contaminants	1–30
Sensor 12	Carbon monoxide and Combustible gas	100–10,000
Sensor 13	LP gas	–
Sensor 14	Ozone gas	–
Sensor 15	Solvent vapors	50–5000
Sensor 16	NH_3, NOx, Alcohol, Benzenes, smoke, CO_2NH_3	10–1000

3.8. Microbial quality

Table 6 summarizes the results of the microbial analysis, including total plate count, total yeast and molds, and sanitary indicator bacteria (coliforms and *E. coli*) in the green tea infusions. The initial total plate count for the untreated control samples (CB) was 47.8 CFU/g. HPP treatment led to a significant reduction in the total plate count, with over 90 % of the microorganisms inactivated. However, no statistically significant differences were observed in total plate count between the HPP-treated samples and the pasteurized samples ($p > 0.05$). The microbial reduction increased with both higher pressures and longer processing durations. Specifically, the CB600:10 treatment was the most effective in achieving microbial inactivation, with total plate counts of 0.00 CFU/g, similar to the HB + Ps treatment (0.3 CFU/g). This suggests that CB600:10 represents the optimal treatment for microbial safety in cold-brewed green tea.

In comparison, the CB + Ps treatment resulted in a total plate count of 1.8 CFU/g, indicating that pasteurization was less effective than HPP at 600 MPa in reducing microbial loads in cold-brewed tea. This highlights the superior microbial inactivation capabilities of HPP at higher pressures and extended durations.

Yeasts, molds, and coliforms were only detected in the untreated CB samples, demonstrating that HPP applied at all tested pressures (300, 450, and 600 MPa) effectively inactivated these microorganisms. *E. coli* was not detected in any of the samples, which indicates that HPP treatment, regardless of pressure or duration, ensured the absence of this harmful pathogen.

Consequently, all tea samples treated with HPP complied with Thailand's regulatory standards as specified in the Notification of the Ministry of Public Health (No. 196) B.E. 2543 (2000), which mandates that coliforms must be <2.2 MPN/100 mL, *E. coli* and other pathogenic microorganisms must be absent, and yeast and molds must not be present. These findings demonstrate the effectiveness of HPP in ensuring the microbial safety of cold-brewed green tea.

3.9. Changes in catechins, TPC, and antioxidant activity during in vitro digestion

The previous section identified CB600:5 as the optimal processing condition for bottled cold-brew green tea due to its effectiveness in microbial inactivation while retaining quality characteristics. This section investigates the changes in catechins, total phenolic content (TPC), and antioxidant activity during *in vitro* digestion, revealing distinct patterns in their behavior.

Table 6
Total plate count, total yeast and molds, coliforms, and *Escherichia coli* of cold-brewed green tea under different treatments: Cold Brew (CB), Cold Brew with HPP (CB300:5, CB300:10, CB450:5, CB450:10, CB600:5, CB600:10), Cold Brew and Hot Brew with Pasteurization at 65 °C for 30 min (CB + Ps and HB + Ps).

Treatment	Total plate count (CFU/mL)	Total yeast and molds (CFU/mL)	Coliforms (CFU/mL)	*E. coli* (CFU/mL)
CB	47.8 ± 6.9^a	1.5 ± 1.9^a	1.5 ± 1.9^a	0
CB300:5	3.5 ± 0.6^b	0^b	0^b	0
CB300:10	1.8 ± 1.5^b	0^b	0^b	0
CB450:5	1.8 ± 1.4^b	0^b	0^b	0
CB450:10	1.5 ± 0.6^b	0^b	0^b	0
CB600:5	0.8 ± 1.0^b	0^b	0^b	0
CB600:10	0.0 ± 0.0^b	0^b	0^b	0
CB + Ps	1.8 ± 1.5^b	0^b	0^b	0
HB + Ps	0.3 ± 0.5^b	0^b	0^b	0

Values expressed mean ± SD ($n = 3$). Data of different alphabets were different with statistical significance ($p < 0.05$). Regulatory limits from Notification of the Ministry of Public Health of Thailand (No. 196) B.E. 2000: Tea in Thailand: <2.2 MPN/100 mL for coliforms, negative for *E. coli*, and negative for pathogenic microorganisms, and negative for yeast and molds.

Fig. 3 illustrates the stability of individual catechins in the CB600:5 infusion during the digestive process. The digestion process resulted in varying changes across the different catechin isomers. Catechin (C) was not detected at any point during digestion. Although gallocatechin (GC) was initially below the detection limit, its concentration increased significantly during digestion.

Epigallocatechin (EGC) and epigallocatechin gallate (EGCG) showed significant degradation during digestion, with recovery rates of 28 % and 58 %, respectively (Fig. 3f and h). In contrast, other catechins such as catechin gallate (CG), caffeine (*CF*), gallocatechin gallate (GCG),

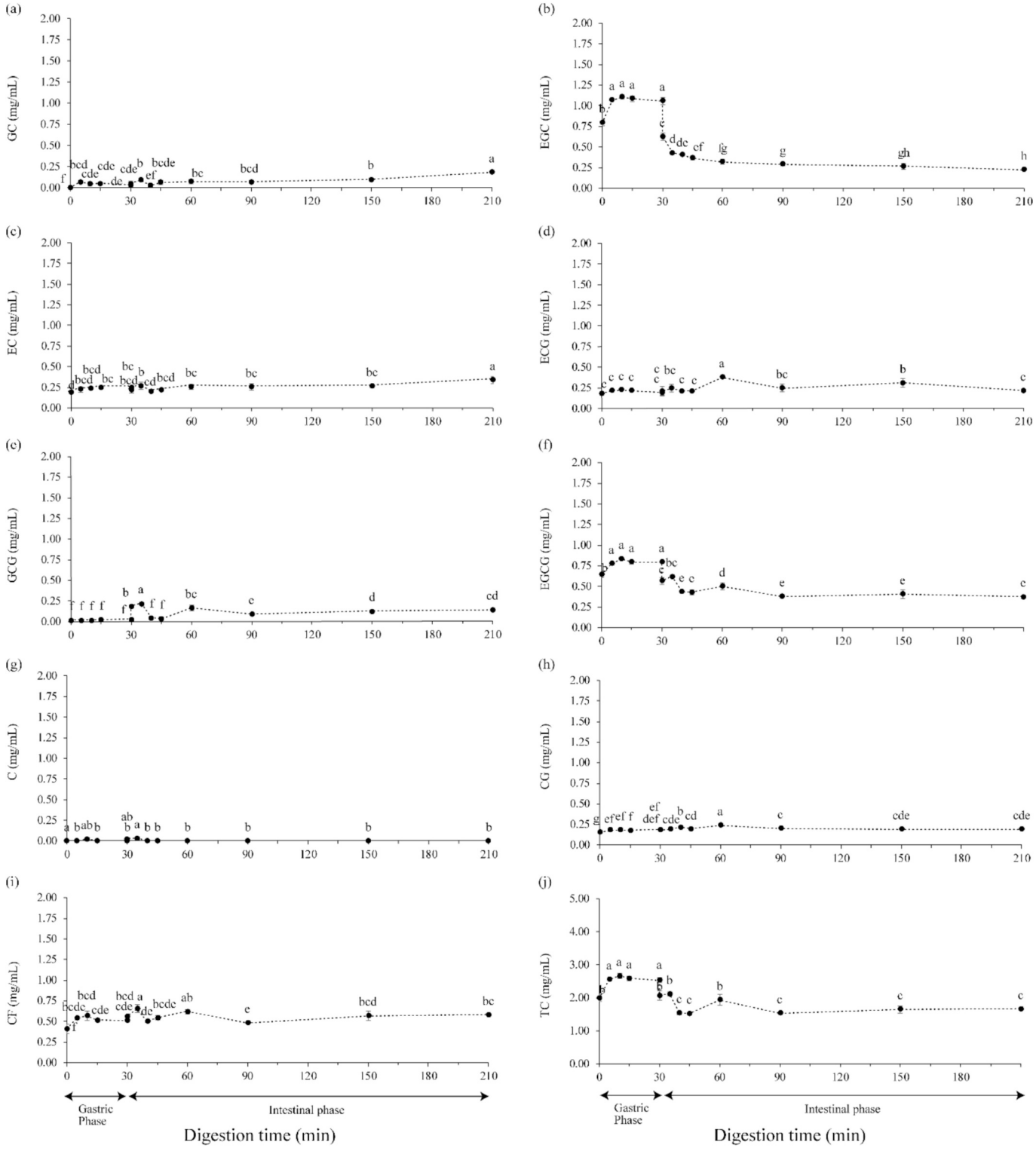


Fig. 3. Changes in individual catechin concentrations during *in vitro* gastrointestinal digestion of cold-brewed green tea treated with HPP 600 MPa for 5 min (CB600:5). (a) EGC; (b) EC; (c) GCG; (d) C; (e) Caffeine; (f) EGC; (g) ECG; (h) EGCG; (i) CG; (j) Total catechins. Values expressed mean ± SD ($n = 3$). Data of different alphabets were different with statistical significance ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

epicatechin (EC), and gallocatechin (GC) demonstrated significant concentration increases ($p < 0.05$), ranging from 16 % to 80 %, in descending order. The overall recovery rate for total catechins (TC) was 83 % (Fig. 3j). Statistical analysis confirmed significant decreases in EGC, EGCG, and TC concentrations compared to their initial levels at 0 min ($p < 0.05$), while significant increases were observed for CG, GCG, *CF*, EC, and GC ($p < 0.05$).

A slight increase in ECG concentration was observed, but this change was not statistically significant. The degradation of EGC and EGCG during digestion is likely due to oxidation, rearrangement, and epimerization, particularly accelerated by the presence of dissolved oxygen and a higher pH in the synthetic intestinal fluid (Governa, Manetti, Miraldi, & Biagi, 2022; Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007). These findings are consistent with prior studies indicating that EGCG remains stable in gastric acid but rapidly degrades during the intestinal phase.

On the other hand, catechins like GC, EC, and GCG showed slow increases during digestion. This suggests that enzymatic activity may make them more soluble and available to the body. ECG levels fluctuated during digestion, but the final content remained similar to the initial levels, indicating its relative stability. Shim et al. (2012) found that EC and ECG had higher recovery rates than EGC and EGCG, which is consistent with the fact that EC and ECG are more stable. The stability of EGCG diminished with elevated pH and temperature, resulting in accelerated oxidation and degradation, while epimerization to GCG intensified. These results highlight EGCG's sensitivity to environmental conditions, confirming its susceptibility to degradation (Xu, Yu, & Zhou, 2019).

The difference in digestive stability between catechins, such as EGC and EGCG *versus* EC and ECG, is attributed to their molecular structure. The presence of three adjacent hydroxyl groups (3′, 4′, and 5′—the pyrogallol moiety) in the B ring of EGC and EGCG makes them more prone

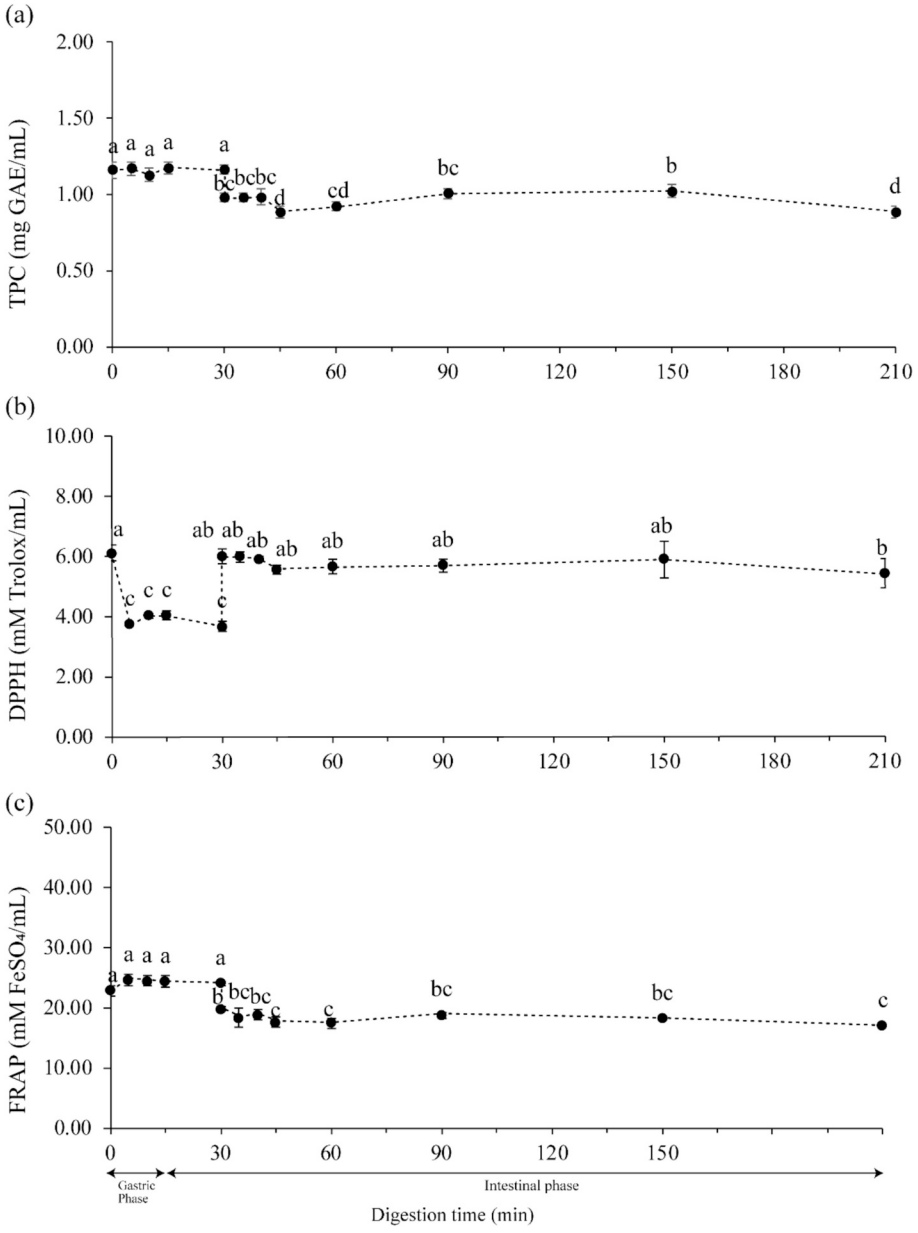


Fig. 4. Changes in TPC and antioxidant activities of cold-brewed green tea treated with HPP 600 MPa for 5 min (CB600:5) during *in vitro* gastrointestinal digestion. (a) Total phenolic content (TPC); (b) DPPH radical scavenging activity; (c) Ferric reducing antioxidant power (FRAP). Values expressed mean ± SD (n = 3). Data of different alphabets were different with statistical significance ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to forming semiquinone free radicals at near-neutral pH after donating a proton, leading to greater vulnerability (Green et al., 2007). Conversely, EC and ECG were less prone to auto-oxidation due to the dihydroxy (catechol) structure in their B ring, making them more resilient during simulated digestion (Green et al., 2007).

Interestingly, CG and caffeine remained stable throughout the digestive process, indicating their resilience to *in vitro* digestion. The detection of C in the digested infusion suggests that catechins undergo transformation during digestion. This observation is consistent with existing research on how substances metabolize in the body. The complex dynamics of catechin metabolism—driven by oxidation, epimerization, and likely influenced by dissolved oxygen and intestinal pH—significantly affect their bioavailability during digestion (Green et al., 2007; Peters, Green, Janle, & Ferruzzi, 2010). Overall, the TC content increased during the gastric phase but subsequently decreased in the intestinal phase. This pattern suggests that catechin availability is not constant throughout the digestive tract and is influenced by both digestive conditions and metabolic transformations.

Fig. 4 illustrates the stability of TPC, DPPH radical scavenging activity, and FRAP during *in vitro* digestion. Recovery rates for FRAP, TPC, and DPPH ranged from 70 % to 90 %. TPC and FRAP exhibited similar trends, both showing a significant decrease during the gastric phase followed by a sharp drop upon entering the intestinal phase ($p < 0.05$). Specifically, TPC remained stable during the gastric phase but then significantly decreased in the intestinal phase.

The DPPH radical scavenging activity showed a distinct pattern. It declined sharply immediately after the addition of pepsin but subsequently increased in the intestinal phase, with a slight decrease at the end of digestion. This initial decline is due to DPPH being unstable in acidic conditions, since it is most stable between pH 4 and 8 (Ferri, Gianotti, & Tassoni, 2013). The use of the DPPH assay in an acidic environment may lead to false-positive results, as protonation decreases ionization potential and disrupts ionization equilibrium, weakening the measured antioxidant activity (Siddeeg, AlKehayez, Abu-Hiamed, Al-Sanea, & Al-Farga, 2021).

FRAP values showed a slight increase at the start of the gastric phase but dropped substantially at the onset of intestinal digestion, with minimal changes thereafter. Notably, the overall trends of TPC and FRAP mirrored each other and aligned with the changes observed in EGCG (Figs. 4a, c, and 3h) during *in vitro* digestion. Despite fluctuations, the final concentrations of TPC, DPPH, and FRAP were comparable to their initial levels, indicating the relative stability and high recovery of TPC and antioxidant capacity during digestion.

These findings underscore the crucial influence of digestion on the bioavailability and health benefits of green tea catechins, supporting existing studies on flavonoid bioavailability. Further research is needed to precisely elucidate the specific mechanisms driving these alterations, especially considering the complex interactions between catechins, pH changes, and enzymatic processes in the digestive tract.

3.10. Feasibility of high-pressure processing (HPP) for industrial-scale applications and future directions

HPP is increasingly feasible for the industrial-scale production of beverages and plant-based foods; however, several technical and economic constraints must be considered. Commercial HPP systems with large pressure vessels (like 525 L) can process up to about 3000 L h^{-1} under the best conditions, such as ~10 cycles per hour at 600 MPa. This shows that medium-to-large throughput is possible with current technology (Houška, Silva, Evelyn Buckow, Terefe, & Tonello, 2022). Nevertheless, because HPP is inherently a batch-wise rather than continuous process, overall productivity remains lower than that of conventional thermal pasteurization, and scaling up often requires the installation of multiple parallel units, thereby increasing capital investment and operational complexity.

The cost of industrial HPP equipment remains a major barrier, with capital expenditures typically ranging from US $1.5 to 5 million (Nabi et al., 2021), in addition to higher operational expenses associated with energy consumption and equipment maintenance. Packaging requirements also influence feasibility, as HPP generally relies on flexible, pressure-resistant containers or, alternatively, emerging bulk-processing systems followed by aseptic bottling, which may increase material costs or limit packaging options. Despite these challenges, the ability of HPP to maintain superior product quality—particularly the retention of sensory attributes, nutrients, and bioactive compounds—while ensuring microbial safety and extended shelf life provides a strong economic justification for its use in premium or value-added beverage products (Waghmare, 2024). These advantages make HPP especially attractive for functional beverages, such as cold-brewed tea, where quality preservation is a key market driver.

3.11. Practical applications

In line with the industrial feasibility discussed above, high-pressure processing represents a promising non-thermal technology for the production of premium cold-brewed green tea beverages. Under processing conditions of 600 MPa for 5 min, HPP effectively inactivates spoilage and pathogenic microorganisms while preserving desirable quality attributes, including color, natural aroma, catechins, and ascorbic acid, thereby outperforming conventional thermal pasteurization. The high retention of key bioactive compounds (>70 %) observed during simulated digestion further indicates improved nutritional stability and potential health benefits, supporting the value-added positioning of HPP-treated green tea products.

Although the batch nature and higher costs of HPP may limit its application in low-margin beverage markets, the demonstrated quality and functional advantages align well with premium product segments, where consumers are willing to pay for minimally processed, health-oriented beverages. Consequently, the findings of this study support HPP as a viable industrial strategy for cold-brewed green tea production. Future research should focus on long-term shelf-life stability, comprehensive sensory evaluation, and process optimization at larger scales to further enhance commercial viability and cost-effectiveness.

4. Conclusions

This study demonstrates the potential of HPP as an effective non-thermal alternative for preserving cold-brewed green tea. HPP at 600 MPa for 5 min was identified as the optimal treatment, achieving >90 % microbial inactivation while maintaining key quality attributes such as color, ascorbic acid, and catechins. Extending the treatment to 600 MPa for 10 min led to increased nutrient degradation and higher turbidity, suggesting that prolonged exposure may compromise the tea's nutritional and visual quality. HPP caused moderate changes in volatile profiles, which were less pronounced than those observed with thermal pasteurization, indicating that HPP may better preserve natural flavors; however, sensory evaluation is required to confirm these effects. *In vitro* digestion showed that more than 70 % of the total catechins, total phenolic content, and antioxidant activity were still present. Some catechins (GC and EC) even showed better bioavailability, which suggests that HPP-treated tea may still have health benefits. Overall, HPP proved more effective than thermal pasteurization in maintaining both nutritional and microbiological quality, offering a promising approach for producing safe, high-quality beverages. Future work should include long-term storage studies, formal sensory evaluation, *in vivo* nutrient bioavailability assessments, and exploration of synergistic preservation strategies to optimize HPP for commercial-scale applications.

CRediT authorship contribution statement

Natthawuddhi Donlao: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition,

Data curation, Conceptualization. **Chhoryi Sim:** Formal analysis. **Jaspreet Singh:** Writing – review & editing. **Lovedeep Kaur:** Writing – review & editing. **Jinhu Tian:** Writing – review & editing. **Suphat Phongthai:** Writing – review & editing. **Yardfon Tanongkankit:** Writing – review & editing. **Utthapon Issara:** Writing – review & editing. **Chanthima Phungamngoen:** Writing – review & editing. **Wirongrong Tongdeesoontorn:** Writing – review & editing, Resources. **Saranchanok Wonglek:** Writing – original draft, Validation, Investigation, Formal analysis.

Ethics approval

This study did not include any human subjects or animal experiments.

Funding

This research was supported by Mae Fah Luang University through the research center of innovative food packaging and biomaterials unit. The work was also supported by Reinventing University (Grant No. F01-683R-04-045), which has received funding from the Office of the Permanent Secretary of the Ministry of Higher Education, Science, Research and Innovation, Thailand.

Declaration of competing interest

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.

Acknowledgments

The authors would like to express their gratitude to Boonrawd Farm Co., Ltd. and Choke Chamroen Tea Co., Ltd. for providing the tea samples. We sincerely thank Dr. Sunantha Ketnawa for her valuable assistance in improving the manuscript, including refining the abstract and enhancing the discussion. The authors also acknowledge Mae Fah Luang University, Chiang Rai, Thailand, for technical and financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.103473.

Data availability

Data will be made available on request.

References

Akhmazillah, M. F. N., Farid, M. M., & Silva, F. V. M. (2013). High pressure processing (HPP) of honey for the improvement of nutritional value. *Innovative Food Science & Emerging Technologies, 20*, 59–63.

Ali, N., Popović, V., Koutchma, T., Warriner, K., & Zhu, Y. (2020). Effect of thermal, high hydrostatic pressure, and ultraviolet-C processing on the microbial inactivation, vitamins, chlorophyll, antioxidants, enzyme activity, and color of wheatgrass juice. *Journal of Food Process Engineering, 43*(1), Article e13036.

Ananingsih, V. K., Sharma, A., & Zhou, W. (2013). Green tea catechins during food processing and storage: A review on stability and detection. *Food Research International, 50*(2), 469–479.

AOAC International. (2016). *Official methods of analysis* (20th ed.). Rockville, MD: AOAC International (On-line). Method 976.21,.

Bellumori, M., Angeloni, G., Guerrini, L., Masella, P., Calamai, L., Mulinacci, N., & Innocenti, M. (2021). Effects of different stabilization techniques on the shelf life of cold brew coffee: Chemical composition, flavor profile and microbiological analysis. *LWT, 142*, Article 111043.

Chen, X., Wang, P., Wei, M., Lin, X., Gu, M., Fang, W., & Ye, N. (2022). Lipidomics analysis unravels changes from flavor precursors in different processing treatments of purple-leaf tea. *Journal of the Science of Food and Agriculture, 102*(9), 3730–3741.

Chen, X., Zhang, Y., Li, X., Ye, Y., Cheng, H., & Wang, X. (2009). Preliminary study on green tea juice extracted by ultrahigh pressure process. *Transactions of the Chinese Society of Agricultural Engineering, 25*(12), 335–338.

Chiang, S. H., Tsou, M. F., Chang, C. Y., & Chen, C. W. (2020). Physicochemical characteristics, sensory quality, and antioxidant properties of Paochung tea infusion brewed in cold water. *International Journal of Food Properties, 23*(1), 1611–1623.

Ferri, M., Gianotti, A., & Tassoni, A. (2013). Optimisation of assay conditions for the determination of antioxidant capacity and polyphenols in cereal food components. *Journal of Food Composition and Analysis, 30*(2), 94–101.

Gan, R. Y., Li, H. B., Sui, Z. Q., & Corke, H. (2018). Absorption, metabolism, anti-cancer effect and molecular targets of epigallocatechin gallate (EGCG): An updated review. *Critical Reviews in Food Science and Nutrition, 58*(6), 924–941.

Governa, P., Manetti, F., Miraldi, E., & Biagi, M. (2022). Effects of in vitro simulated digestion on the antioxidant activity of different *Camellia sinensis* (L.) Kuntze leaves extracts. *European Food Research and Technology*, 1–10.

Green, R. J., Murphy, A. S., Schulz, B., Watkins, B. A., & Ferruzzi, M. G. (2007). Common tea formulations modulate *in vitro* digestive recovery of green tea catechins. *Molecular Nutrition & Food Research, 51*(9), 1152–1162.

Houška, M., Silva, F. V. M., Evelyn Buckow, R., Terefe, N. S., & Tonello, C. (2022). High pressure processing applications in plant foods. *Foods, 11*(2), 1–12.

Huang, Y., Doh, I. J., & Bae, E. (2021). Design and validation of a portable machine learning-based electronic nose. *Sensors, 21*(11), 3923.

Huang, Y., Sheng, J., Yang, F., & Hu, Q. (2007). Effect of enzyme inactivation by microwave and oven heating on preservation quality of green tea. *Journal of Food Engineering, 78*(2), 687–692.

Jeż, M., Wiczkowski, W., Zielińska, D., Białobrzewski, I., & Błaszczak, W. (2018). The impact of high pressure processing on the phenolic profile, hydrophilic antioxidant and reducing capacity of purée obtained from commercial tomato varieties. *Food Chemistry, 261*, 201–209.

Li, N., Taylor, L. S., Ferruzzi, M. G., & Mauer, L. J. (2012). Kinetic study of catechin stability: Effects of pH, concentration, and temperature. *Journal of Agricultural and Food Chemistry, 60*(51), 12531–12539.

Lin, S. D., Yang, J. H., Hsieh, Y. J., Liu, E. H., & Mau, J. L. (2014). Effect of different brewing methods on quality of green tea. *Journal of Food Processing and Preservation, 38*(3), 1234–1243.

Lin, Y. H., Huang, H. W., & Wang, C. Y. (2022). Effects of high pressure-assisted extraction on yield, antioxidant, antimicrobial, and anti-diabetic properties of chlorogenic acid and caffeine extracted from green coffee beans. *Food and Bioprocess Technology, 15*(7), 1529–1538.

Liu, H., Ueno, S., Shimada, R., & Araki, T. (2019). The effect of high hydrostatic pressure on taste substance distribution in fresh green tea leaves. *High Pressure Research, 39*(2), 408–416.

Lu, J. L., Pan, S. S., Zheng, X. Q., Dong, J. J., Borthakur, D., & Liang, Y. R. (2009). Effects of lipophillic pigments on colour of the green tea infusion. *International Journal of Food Science & Technology, 44*(12), 2505–2511.

Meyer, B. R., White, H. M., McCormack, J. D., & Niemeyer, E. D. (2023). Catechin composition, phenolic content, and antioxidant properties of commercially-available bagged, gunpowder, and matcha green teas. *Plant Foods for Human Nutrition, 78*(4), 662–669.

Nabi, B. G., Mukhtar, K., Arshad, R. N., Radicetti, E., Tedeschi, P., Shahbaz, M. U., & Aadil, R. M. (2021). High-pressure processing for sustainable food supply. *Sustainability, 13*(24), 1–12.

Peters, C. M., Green, R. J., Janle, E. M., & Ferruzzi, M. G. (2010). Formulation with ascorbic acid and sucrose modulates catechin bioavailability from green tea. *Food Research International, 43*(1), 95–102.

Ruan, L., Wei, K., Wang, L., Cheng, H., Wu, L., & Li, H. (2019). Characteristics of free amino acids the quality chemical components of tea under spatial heterogeneity of different nitrogen forms in tea (*Camellia sinensis*) plants. *Molecules, 24*(3), 415.

Šeremet, D., Karlović, S., Vojvodić Cebin, A., Mandura, A., Ježek, D., & Komes, D. (2021). Extraction of bioactive compounds from different types of tea by high hydrostatic pressure. *Journal of Food Processing and Preservation, 45*(9), Article e15751.

Sheng, Y., Sun, Y., Tang, Y., Yu, Y., Wang, J., Zheng, F., & Sun, Y. (2023). Catechins: Protective mechanism of antioxidant stress in atherosclerosis. *Frontiers in Pharmacology, 14*, 1144878.

Shim, S. M., Yoo, S. H., Ra, C. S., Kim, Y. K., Chung, J. O., & Lee, S. J. (2012). Digestive stability and absorption of green tea polyphenols: Influence of acid and xylitol addition. *Food Research International, 45*(1), 204–210.

Siddeeg, A., AlKehayez, N. M., Abu-Hiamed, H. A., Al-Sanea, E. A., & Al-Farga, A. M. (2021). Mode of action and determination of antioxidant activity in the dietary sources: An overview. *Saudi Journal of Biological Sciences, 28*(3), 1633–1644.

Song, Y., Bi, X., Zhou, M., Zhou, Z., Chen, L., Wang, X., & Ma, Y. (2021). Effect of combined treatments of ultrasound and high hydrostatic pressure processing on the physicochemical properties, microbial quality and shelf-life of cold brew tea. *International Journal of Food Science & Technology, 56*(11), 5977–5988.

Tallei, T. E., Fatimawali Niode, N. J., Idroes, R., Zidan, B. R. M., Mitra, S., & Capasso, R. (2021). A comprehensive review of the potential use of green tea polyphenols in the management of COVID-19. *Evidence-based Complementary and Alternative Medicine, 2021*(1), Article 7170736.

Tepsongkroh, B., Thaihuttakij, C., Supawong, S., & Jangchud, K. (2023). Impact of high pressure pre-treatment and hot water extraction on chemical properties of crude polysaccharide extract obtained from mushroom (*Volvariella volvacea*). *Food Chemistry: X, 19*, Article 100864.

Tewari, S., Sehrawat, R., Nema, P. K., & Kaur, B. P. (2017). Preservation effect of high pressure processing on ascorbic acid of fruits and vegetables: A review. *Journal of Food Biochemistry, 41*(1), Article e12319.

Uzuner, S., & Evrendilek, G. A. (2019). Functional responses of cold brewed white tea to high pressure processing. *Journal of Food Process Engineering, 42*(5), Article e13098.
Waghmare, R. (2024). High pressure processing of fruit beverages: A recent trend. *Food and Humanity, 2*, Article 100232.
Wang, L. F., Park, S. C., Chung, J. O., Baik, J. H., & Park, S. K. (2004). The compounds contributing to the greenness of green tea. *Journal of Food Science, 69*(8), S301–S305.
Wang, R., Xu, Q., Yao, J., Zhang, Y., Liao, X., Hu, X., & Zhang, Y. (2013). Post-effects of high hydrostatic pressure on green color retention and related properties of spinach puree during storage. *Innovative Food Science & Emerging Technologies, 17*, 63–71.
Wong, M., Sirisena, S., & Ng, K. (2022). Phytochemical profile of differently processed tea: A review. *Journal of Food Science, 87*(5), 1925–1942.
Wonglek, S., Jaikaew, C., Ogawa, Y., Singh, J., Panpipat, W., & Donlao, N. (2024). Characterization of flours from some underutilized carbohydrate sources of Thailand for potential food applications. *LWT, 199*, Article 116079.
Xu, Y. Q., Yu, P., & Zhou, W. (2019). Combined effect of pH and temperature on the stability and antioxidant capacity of epigallocatechin gallate (EGCG) in aqueous system. *Journal of Food Engineering, 250*, 46–54.
Ye, J. H., Ye, Y., Yin, J. F., Jin, J., Liang, Y. R., Liu, R. Y., & Xu, Y. Q. (2022). Bitterness and astringency of tea leaves and products: Formation mechanism and reducing strategies. *Trends in Food Science & Technology, 123*, 130–143.
Yeo, H., Kim, S. Y., Shahbaz, H. M., Jeong, S. H., Ju, H. I., Jeon, J. H., & Lee, D. U. (2024). Effects of pulsed electric field Pretreatment on black tea processing and its impact on cold-brewed tea. *Foods, 13*(1), 164.
Yu, X., Hu, S., He, C., Zhou, J., Qu, F., Ai, Z., & Ni, D. (2019). Chlorophyll metabolism in postharvest tea (*Camellia sinensis* L.) leaves: Variations in color values, chlorophyll derivatives, and gene expression levels under different withering treatments. *Journal of Agricultural and Food Chemistry, 67*(38), 10624–10636.
Zeng, J., Xu, H., Cai, Y., Xuan, Y., Liu, J., Gao, Y., & Luan, Q. (2018). The effect of ultrasound, oxygen and sunlight on the stability of (−)-epigallocatechin gallate. *Molecules, 23*(9), 2394.
Zhang, L., Cao, Q. Q., Granato, D., Xu, Y. Q., & Ho, C. T. (2020). Association between chemistry and taste of tea: A review. *Trends in Food Science & Technology, 101*, 139–149.
Zhang, W., Huang, D., Liu, Y., Guan, H., Wang, M., Chen, H., & Li, D. (2024). Effects of high pressure processing on structural changes, aggregation, and binding mechanisms of β-lactoglobulin with typical polyphenols. *Food Chemistry, 458*, Article 140265.
Zhang, Y., Yan, K., Peng, Q., Baldermann, S., Zhu, Y., Dai, W., & Shi, J. (2024). Comprehensive analysis of pigment alterations and associated flavor development in strip and needle green teas. *Food Research International, 175*, Article 113713.