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Effects of Water Mineral content and Infusion Parameters on the Chemical Composition of Green and Black Tea Matrices for Kombucha Production

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Abstract

Water is a critical variable in beverage production, particularly due to regional differences in mineral composition. This study systematically investigated how varying levels of water mineral content, combined with controlled infusion temperature and time, influence the chemical profile of sweetened green and black tea matrices. These matrices subsequently served as the base for controlled kombucha fermentations. Our findings demonstrate that water mineral content has a pronounced effect on complex formation, turbidity, and the overall chemical composition of tea, including its polyphenol and protein content. Specifically, hard water containing elevated concentrations of Ca^{2+} and Mg^{2+} promotes extensive complexation with tea-derived polyphenols and proteins. This not only reduces the soluble polyphenol and protein fractions but also increases turbidity and alters colour development. Such changes in the appearance and stability of the tea matrix may also significantly influence the nutritional and biochemical substrate available for subsequent fermentation. Overall, this research highlights the pivotal role of water mineral content in determining the chemical properties of tea infusions prior to kombucha fermentation. These insights underline the importance of considering water chemistry as a key process parameter in kombucha production and provide valuable guidance for the development of Good Manufacturing Practices (GMP) within this rapidly expanding beverage category.

Introduction

Acidic, fermented, and non- or low-alcoholic beverages, such as kombucha and water kefir, have gained substantial prominence in the functional beverage market. This has been driven by increasing consumer interest in health-oriented products [1, 2]. Kombucha has recently experienced a surge in popularity, leveraging the already well-recognised functional and health-promoting properties of traditional tea, to create a value-added derivative [3]. In 2024, the global kombucha market was estimated at US$2.97 billion and is expected to grow to US$4.65 billion by 2029 at a compound annual growth rate (CAGR) of 9.48% [4]. The importance of water quality is a historical tenet of tea preparation, dating back to Lu Yu's *The Classic of Tea* (758 AD), which ranked water sources by their suitability for brewing [5]. As the primary ingredient, water plays a dual role: it acts as the solvent for tea extraction and the medium for microbial metabolism. Its mineral composition can significantly influence the extraction of bioactive compounds from tea leaves *(Camellia sinensis)*, thereby affecting the chemical profile and sensory characteristics of the resulting beverage [5, 6]. Despite growing commercial interest, however, the influence of water mineral content on fermentation behaviour and product quality remains understudied and poorly understood. This stands in contrast to beer production, where water chemistry is well-characterised and routinely integrated into standard brewing protocols [7]. Beyond extraction, water minerals directly influence microbial activity, shaping metabolite production and flavour development during fermentation [5, 8–10].

Water quality varies substantially across regions due to local hydrogeology. As water is often used untreated in kombucha production, understanding its potential impact on process performance and product quality is essential. While the water used in food and beverage production must typically meet

national drinking water standards, such as the German Drinking Water Ordinance [11], these regulatory thresholds prioritise safety rather than the optimal chemical conditions for beverage quality. Dissolved ions and trace metals can act as catalysts in oxidation reactions, altering the flavour and stability of tea infusions [6, 12]. Elevated Cu^{2+} or Fe^{3+} concentrations in particular can accelerate oxidation [13, 14] adversely affecting flavour, astringency, and colour. Likewise, reductions in soluble Ca^{2+} and Mg^{2+} may influence turbidity [15] and mouthfeel [5].

Tea leaves naturally accumulate minerals from the soil during growth, with concentrations varying according to leaf age, cultivar, and processing conditions. These intrinsic minerals, combined with those present in the brewing water, collectively shape the mineral environment of the tea infusion. Functionally, this mineral composition may influence not only the nutritional value of the infusion but also its physicochemical and sensory properties, including colour, astringency, and stability [16]. Given that many plant-derived compounds can impact microbial metabolism, the tea infusion process is expected to significantly influence subsequent fermentation behaviour.

This study aimed to investigate the influence of water mineral composition and infusion parameters, specifically temperature and time, on the chemical profile of sweetened green and black tea infusions prepared for kombucha production. Green and black tea were selected to represent the two most widely used substrates in kombucha brewing, allowing for a comparative assessment across contrasting oxidation levels and chemical compositions, particularly with respect to polyphenols. Tea matrices were prepared using waters of distinct mineral content and subjected to varying time-temperature infusion regimes. A comprehensive suite of physicochemical and biochemical analyses was performed to determine how water mineral composition alters key chemical attributes of the infusion prior to fermentation. The influence of these matrices on subsequent kombucha fermentation will be reported separately.

Materials and Methods

Water Qualities

To investigate the impact of water quality on the extraction behaviour of key tea components, unfermented tea infusions prepared with three different types of water were analysed and compared. A reverse osmosis permeate served as deionised water (DI) representing the low mineral reference, while the high-salt water (HS) was formulated by supplementing tap water with salts to match the maximum mineral content permitted under the German Drinking Regulation [11]. Berlin tap water (TW) was included as a practically relevant reference, representative of the water conditions commonly encountered in regional kombucha production. The relevant parameters of the water types used are given below in Table 1.

Tea Preparation and Infusion Studies

To establish matrix-specific infusion conditions, 12 tea variations were prepared. Two types of tea (*Camellia sinensis*), green tea (China sencha, Ökotopia GmbH) and black tea (Ostfriesische Brokenmischung, Ökotopia GmbH), were infused using a tea strainer. For green tea (GT), two infusion temperatures (75°C and 100°C) and one infusion time (10 min) were applied, while black tea (BT) was infused for two different durations (5 and 10 minutes) at 100°C. Each temperature-time combination was prepared using all three water types with different levels of mineral content. Subsequently, an in-house kombucha recipe was applied, consisting of 8 g/L tea leaves, 40 g/L sucrose (Carl Roth GmbH, Germany) and 20 g/L glucose (AppliChem GmbH, Germany). For each sample, the corresponding treated water was added to a beaker containing the sugars and tea leaves in a strainer. The beakers were covered with aluminium foil and allowed to steep at the aforementioned temperature and time. After each steeping time, the tea strainer was promptly removed, and the sweetened tea infusion was transferred to a laboratory bottle with a lid and cooled in a water bath.

Analytical Methods

Determination of pH Value

The pH value was determined using a laboratory pH meter (Type 766, Knick Elektronische Messgeräte GmbH & Co. KG, Germany).

Total Polyphenol Content (TPPC)

Total polyphenol content (TPPC) was quantified spectroscopically, using the Folin-Ciocalteu method. The filtered, 10-fold diluted kombucha samples were mixed with 200 µL of 10% (v/v) Folin-Ciocalteu reagent (Sigma-Aldrich, USA). Then, 700 µL of a 700 mM Na_2CO_3 solution (Merck KGaA, Germany) was added to the mixture, which was then left to incubate at room temperature for a period of two hours. After vortexing, 200 µL of each reaction mixture was transferred to a 96-well plate. The absorbance was measured at 750 nm using a UV-vis spectrometer (BioTek Instruments, USA), and TPPC was calculated based on a standard calibration curve prepared with gallic acid (Sigma-Aldrich, USA).

Antioxidant Capacity (AC)

Antioxidant capacity (AC) of fermented kombucha samples was measured using the Trolox Equivalent Antioxidative Capacity (TEAC) assay, which quantifies antioxidant activity based on the reduction in colour intensity of the $ABTS^{\bullet+}$ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation [17]. The Trolox (Thermo Fisher Scientific Inc., USA) standard dilution series (20 µL), prepared with KH_2PO_4 buffer and Trolox working solution (0.225 mM), was pipetted into a 96-well plate in duplicate, followed by 100-fold diluted kombucha samples after filtration, in triplicate (equal volume). After adding 200 µL of ABTS reagent (Alfa Aesar, USA) using a multichannel pipette, the plate was incubated in the dark for about 6 min at 25°C, and the absorbance at a wavelength of 734 nm was measured using a tempered

UV-vis spectrophotometer (BioTek Instruments, USA). The decrease in absorbance reflects the extent of interaction between the antioxidant compounds and the $ABTS^{\bullet+}$ radical cation. A standard curve generated from the Trolox dilutions was used to determine the Trolox equivalent antioxidant concentration in the kombucha samples.

Total Protein Content (TPC)

Protein concentration was determined using the Bradford assay, a dye-binding method based on the interaction of Coomassie Brilliant Blue G-250 with proteins [18]. Bovine serum albumin (BSA, Th. Geyer GmbH & Co. KG, Germany) was used as the standard, with a stock solution of 1 mg/mL prepared in distilled water and stored at -20°C. The exact concentration of BSA was verified by measuring absorbance at 280 nm, using a molar extinction coefficient of 0.66 L/mol*cm for a 1 mg/mL solution. Samples were diluted 1:100 in deionised water to fall within the assay's detection range. Bradford reagent (Bio-Rad Laboratories, USA) was diluted 2.5-fold with deionised water, and 100 µL was added to 100 µL of each diluted sample or standard in a 96-well plate. Following incubation for 5 to 60 minutes, absorbance was measured at 590 nm and 450 nm using a tempered UV-vis spectrophotometer (BioTek Instruments, USA). A calibration curve was generated using the ratio of absorbance at 590 nm to 450 nm, and protein concentrations of unknown samples were calculated using the linear equation of the curve.

Free Amino Nitrogen Content (FAN)

Free amino nitrogen (FAN) concentration in kombucha samples was determined using the ninhydrin spectrophotometric method. Samples were initially diluted 1:10 using deionised water to ensure accurate measurement within the assay's linear range. Reaction mixtures were prepared in triplicate by combining 250 µL of the sample or standard solution with 125 µL of the dye reagent (prepared from 1 g/L disodium hydrogen phosphate dodecahydrate (Carl Roth GmbH + Co. KG, Germany), 0.6 g/L potassium dihydrogen phosphate (Carl Roth GmbH + Co. KG, Germany), 0.05 g/L ninhydrin (Merck KGaA, Germany), and 0.03 g/L fructose (Carl Roth GmbH + Co. KG, Germany), buffered to a pH of 6.6–6.8). The reaction tubes were sealed to prevent evaporation and incubated by boiling in a Heating block-ThermoMixer (DITABIS AG, Germany) for 16 minutes. The reaction was quenched, and the purple chromophore was developed by adding 625 µL of a dilution solution (containing 0.02 g/L potassium iodide (Merck KGaA, Germany) and 40% ethanol (VWR International, USA)). The tubes were then rapidly cooled to 20°C using a water bath. After vortexing, 200 µL of each reaction mixture was transferred to a 96-well plate. Absorbance was measured at 570 nm using a tempered UV-vis spectrophotometer (BioTek Instruments, USA) using water as a blank. The FAN concentration was calculated using a calibration curve generated from standard solutions containing glycine, where the detected chromophore's absorbance is proportional to the alpha amino nitrogen content [19].

Colour Measurement

Colour analysis was conducted using the CIELAB uniform colour space method using a CR-5 bench-top colourimeter and the SpectraMagic™NX software (Konica Minolta Business Solutions Deutschland GmbH, Germany). In this system, L* indicates lightness, a* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis.

Turbidity Analysis

Turbidity was measured using a LabScat photometer (Sigrist-Photometer AG, Switzerland) at a wavelength of 690 nm, in accordance with standard measurement principles. The device utilises a measurement angle of 90° ± 2.5° with a measurement wavelength of 690 ± 30 nm. Samples were placed in sealed measurement cuvettes, and turbidity values were obtained following the manufacturer's instructions. Results were expressed in EBC Formazin units. Turbidity was assessed based on the forward-scattered light (measurement angle 25°), as well. Standardised cuvettes were employed throughout to ensure consistency and accuracy across measurements. Turbidity classifications ranged from "bright to clear" (< 1 EBC) to "increasing turbidity intensity" (> 2 EBC). Measurements were performed in triplicate for precision.

Elemental Analysis

Elemental analysis (Ca^{2+}, Cu^{2+}, Fe^{3+}, Mg^{2+} and Mn^{2+}) was performed according to the DIN EN ISO 17294-2 (E29) 2017-01 method, using inductively coupled plasma mass spectrometry (ICP-MS) (ICPMS-2030, Shimadzu Deutschland GmbH, Germany).

Statistical Analysis

The results of the infusion studies were statistically analysed using XLSTAT software version 2024.2.1 (Addinsoft, France). The normality of the data was checked using a Shapiro-Wilk test ($p < 0.05$). One-way ANOVA with post hoc Duncan`s multiple range test (MRT; $p < 0.05$) was used to compare the samples after different treatments. One-way ANOVA with post hoc Duncan's multiple range test ($p < 0.05$) was used to compare the samples after different treatments. Significantly different mean values were indicated using distinct superscript letters. Green tea and black tea infusions were analysed using separate ANOVA models (temperature × water quality, with interaction) for all measured parameters; tea type was therefore not tested directly except at the single condition shared by both teas (100°C, 10 min), where a supplementary two-way ANOVA (tea type × water quality) was performed for TPPC, AC, TPC, pH, and CIELAB colour parameters (L*, a*, b*). Elemental (mineral) content data were determined in duplicate ($n = 2$) for most conditions, which was considered insufficient for reliable ANOVA and post hoc testing; these data are therefore presented and discussed descriptively, without inferential statistical comparison.

Results and Discussion

Mineral content in different tea matrices

Table 2 presents the concentrations of Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+} and Cu^{2+} in green tea and black tea infusions prepared with three different water types, deionised water, tap water and high salt water, under varying infusion temperatures and times. As elemental analyses were performed in duplicate, the differences discussed below are presented as observed/numerical trends rather than statistically tested effects (see Methods). While the baseline composition of the source water clearly influenced initial mineral levels, the tea infusion process itself substantially altered the final elemental composition. Mineral analysis via ICP-MS confirmed that deionised water yielded the lowest post-infusion concentrations of Ca^{2+}, Mg^{2+}, and other micronutrients such as Cu^{2+} and Fe^{3+}, highlighting the strong leaching potential of tea leaves.

The use of deionised water infusions serves as a base line to demonstrate the intrinsic quality and quantity of the ions extracted from the tea leaves. As shown in Tables 1 and 2, the tea prepared using deionised water consistently yielded higher concentrations of all measured elements. The leaf material is the primary source of Mg^{2+}, Cu^{2+}, Fe^{3+} and Mn^{2+}, as teas prepared with deionised water were found to have concentrations approximately equivalent to or higher than the baseline found in tap water before extraction. While only minor discrepancies were observed between the tea varieties and the extraction conditions, a pronounced divergence was noted in Mg^{2+} extraction yield, with black tea releasing three to four times higher Mg^{2+} into the solution than green tea. These results align with Mailik et al. who note that mineral concentrations in stimulant beverages vary based on the age of the plant at harvest, genetics, and soil conditions [20].

The observed reduction in measurable Ca^{2+} concentrations, averaging 22% in green tea and 38% in black tea infusions is consistent with precipitation and complexation phenomena driven by high mineral content in tap water and high-salt water. Ca^{2+} solubility is governed by the hydrogen carbonate (HCO_3^-) equilibrium, heating shifts the balance toward the thermal decomposition of bicarbonate into carbonate. This promotes the precipitation of calcium carbonate ($CaCO_3$), a well-established precursor of tea scum [21]. Extended infusion time promoted marginal increases in complexation, as evidenced by the reduction in Ca^{2+} from 2.0 to 1.5 mg/L in black tea infusions made with deionised water between 5 and 10 minutes. This is further confirmed by the strong positive correlation ($r = 0.98 \pm 0.02$, $p < 0.01$) reported by Xu et al., suggesting that higher initial Ca^{2+} levels in brewing water amplify the extent of Ca^{2+} depletion during brewing, as increasing Ca^{2+} concentrations enhance interactions with tea matrix components, promoting the formation of insoluble or bound calcium species [12]. Beyond inorganic precipitation, the apparent loss of Ca^{2+} is further driven by the extraction of tea-derived proteins, catechins, and organic acids, which act as natural scale inhibitors. Li et al. found that this chelation prevents the ions from participating in scale-formation reactions, effectively "locking" them away [15]. This indicates that increasing concentrations of Ca^{2+} in the brewing medium enhance interactions with tea matrix components, promoting the formation of insoluble or bound calcium species and thus lowering the measurable concentration of Ca^{2+} in the final infusion. Tea proteins supply carboxylate groups that chelate Ca^{2+}, preventing its reaction with CO_3^{2-}, while the B-rings of catechins provide complexation sites for both Ca^{2+} and Mg^{2+}, and organic acids form stable five-membered chelate rings

with these cations, effectively preventing hardness ions from participating in scale-formation reactions and reducing their measurable concentration in the final infusion [15]. Ca^{2+} is also fixed within the leaf matrix through complexation with cell wall pectin, retarding the solubilisation of extractable compounds, while tea polyphenols partially retain Ca^{2+} and Mg^{2+} in the tea residue [22]. Elevated mineral content increases water cluster size, reducing the surface area available for solute diffusion and further limiting extraction efficiency [22]. These combined mechanisms, inorganic precipitation, organic complexation, and reduced extraction efficiency, collectively account for the substantial Ca^{2+} losses observed under high mineral content conditions. The Ca^{2+} and Mg^{2+} levels measured here fall within ranges reported in the literature for green tea and black tea infusions [23, 24], affirming the reproducibility of mineral concentrations under controlled infusion parameters.

The extraction and final concentration of Mg^{2+} in tea infusions demonstrated a distinct dependence on both tea type and water composition. Black tea consistently yielded substantially higher Mg^{2+} concentrations in deionised water infusions, ranging from 6.0 to 6.3 mg/L, compared to 1.6 to 1.8 mg/L in green tea under comparable conditions, indicating that black tea processing promotes the enhanced extractability of Mg^{2+} from the leaf matrix. This is likely attributable to the use of physiologically mature leaves in black tea production, which naturally accumulate higher elemental concentrations [25, 26]. It should be noted that contrasting findings exist, with some studies reporting higher Mg^{2+} content in certain green teas, attributed to chlorophyll structure and processing variations [27, 28]. The data obtained here support higher Mg^{2+} extractability in black tea across all water types and infusion conditions, suggesting that leaf maturity and oxidative processing are the dominant determinants in this context. A marginal reduction in Mg^{2+} was observed with extended infusion time in black tea deionised water infusions, decreasing from 6.3 mg/L at 5 minutes to 6.0 mg/L at 10 minutes, consistent with progressive complexation or partial re-adsorption onto the leaf matrix under prolonged extraction conditions. Higher infusion temperatures appeared to enhance Mg^{2+} complexation, particularly in high salt water, where green tea infusions showed a reduction from 28.0 mg/L at 75°C to 24.5 mg/L at 100°C, suggesting that elevated temperatures promote metal-ligand interactions under high ionic strength conditions.

When brewed with tap water, both tea types exhibited a net increase in Mg^{2+} concentration, with black tea reaching particularly high values of up to 7.2 mg/L net increase at 100°C for 10 minutes. This was notably higher than the net increase observed in deionised water under comparable conditions. This counterintuitive finding may reflect the moderately elevated ionic strength and pH of tap water relative to deionised water, which could enhance Mg^{2+} solubilisation from the leaf matrix, though further investigation is needed to confirm this mechanism. High salt water introduced more complex interactions: while black tea still showed a net Mg^{2+} increase of 2.5 to 2.7 mg/L, this was considerably lower than in tap water, suggesting that the high mineral background creates competing complexation equilibria that limit net Mg^{2+} release. Most notably, green tea brewed in high salt water at 100°C resulted in a net reduction of soluble Mg^{2+} (-2.5 mg/L) compared to the source water, underscoring the critical role of water hardness in modulating final soluble Mg^{2+} content and its capacity to drive net removal of the ion from solution. This behaviour is consistent with the principles of solid-liquid extraction, where

solubility and the presence of complexing agents govern extraction efficiency [29], and aligns with the characterisation of Mg^{2+} as a moderately extractable element from tea leaves, with reported efficiencies of 20–55% [23, 24].

It should also be noted that sucrose (40 g/L) and glucose (20 g/L) were added to the tea matrices prior to analysis, as is standard in kombucha substrate preparation. At these concentrations, sugar addition is unlikely to substantially alter ionic activity coefficients or divalent cation complexation equilibria and is therefore not expected to confound the mineral measurements reported here.

Mn^{2+} concentrations exceeded the upper limit of the calibration range and were therefore not validly quantifiable (n.q.) in green tea infusions across all conditions, and in black tea infusions prepared at 10 minutes. The consistently high Mn^{2+} extraction in green tea infusions, reflected by n.q. values across all conditions, is consistent with the well-established status of tea as a rich dietary source of manganese [30, 31], with extractability further influenced by tea type, leaf maturity, and infusion duration [23, 29]. The pattern observed in black tea infusions is particularly notable: at 5 minutes, Mn^{2+} was quantifiable across all three water types (approximately 1.0–1.1 mg/L), whereas at 10 minutes concentrations exceeded the calibration range, indicating continued substantial leaching between these time points consistent with diffusion-limited extraction from the leaf matrix. Water type did not appear to substantially modulate Mn^{2+} extraction under the conditions tested, as quantifiable black tea infusion values were comparable across deionised water, tap water, and high-salt water infusions at 5 minutes. Targeted quantification of black tea infusions at 5 minutes revealed Mn^{2+} levels of approximately 1.0 mg/L, which substantially exceeds both the German Drinking Water Ordinance limit of 0.05 mg/L [11] and the World Health Organization health-based guideline of 0.4 mg/L [32], though it remains well below the tolerable daily intake (TDI) of 8 mg/day set by EFSA [33]. These concentrations are consistent with the upper end of published ranges for tea infusions and reinforce the relevance of tea as a nutritionally significant dietary source of manganese [31, 34]. It should be noted that the n.q. designation reflects an analytical limitation rather than absence of the ion, and future work should employ appropriately extended calibration ranges to fully characterise Mn^{2+} dynamics across all conditions.

Fe^{3+} levels remained generally low across all infusions. Lower Fe^{3+} values, relative to the source water, observed in several conditions indicate that Fe^{3+} was sequestered during infusion. This pattern is consistent with the propensity of tea polyphenols to form insoluble complexes with Fe^{3+} [35] and suggests that, under these conditions, polyphenol-mediated removal dominated over any extraction from the leaf matrix. This was most pronounced in high-salt water, where both green tea and black tea infusions showed a substantial net reduction in Fe^{3+} content (approximately − 0.35 to -0.42 mg/L) relative to the source water, which contained 0.45 mg/L Fe^{3+} (Table 1). Tap water infusions, with a source Fe^{3+} concentration of 0.01 mg/L, showed negligible net change. In green tea infusions prepared with deionised water, a marginal positive extraction was noted at 75°C (0.007 mg/L), while concentrations fell below the Limit of Quantitation (LOQ: 0.0003 mg/L) at 100°C, suggesting that elevated temperature promotes the formation of insoluble polyphenolic complexes that limit Fe^{3+} solubility, consistent with reports that temperatures exceeding 60°C can reduce Fe^{3+} transfer rates

through this mechanism [24]. Black tea in deionised water showed modest extraction at 100°C (0.012 mg/L at 5 min; 0.004 mg/L at 10 min), with the reduction between time points consistent with progressive complexation or precipitation under prolonged infusion conditions. The Fe^{3+} concentrations measured in green tea-deionised water infusions are at the lower boundary of, or below, the trace range typically reported for green tea infusions (0.02–0.17 mg/L) [31, 36], likely reflecting the near-absence of Fe^{3+} in deionised source water and active polyphenol-mediated sequestration. For black tea prepared with deionised water, values of 0.003–0.012 mg/L fall slightly below the reported range for Indian black tea but remain close to the lower boundary of the 0.03–0.23 mg/L range reported by Fernández et al.

The Cu^{2+} concentration in green tea infusions prepared with deionised water aligns well with the reported range for green tea [37]. For black tea infusions, the measured values for deionised water (0.028–0.033 mg/L) fall below the lower boundary of ranges reported for both Indian and Chinese black tea infusions [37], likely reflecting the near-absence of Cu^{2+} in deionised water source water (0.00 mg/L; Table 1) and the influence of water chemistry on extraction efficiency.

Cu^{2+} levels exhibited divergent trends mediated by water chemistry. In deionised water, where source water Cu^{2+} was absent (Table 1), measured infusion concentrations are most plausibly attributable to extraction from the leaf matrix. Green tea yielded higher Cu^{2+} concentrations than black tea across all deionised water conditions (0.058–0.063 mg/L versus 0.028–0.033 mg/L), suggesting a greater leaf-derived contribution. However, without dry-leaf profiling, definitive source attribution and quantification of extraction efficiency remain beyond the scope of this study.

Tap water, with a source water Cu^{2+} of 0.01 mg/L, showed only a marginal net increase in infusion concentrations. A portion of the extracted Cu^{2+} may exist as Cu-polyphenol complexes [38], though whether the moderate mineral content of tap water relative to high-salt water influenced the balance between soluble and insoluble complex formation cannot be determined from the present data. In high-salt water tea infusions, despite a source water Cu^{2+} concentration of 2.00 mg/L (Table 1), infusion concentrations were considerably lower (0.083–0.209 mg/L), consistent with substantial net removal. This likely reflects the formation of insoluble copper-polyphenol complexes [39] or copper-carbonate precipitates under the elevated ionic strength and alkalinity of high-salt water [38]. McDonald et al. found that Cu^{2+} precipitates more readily than other metals (like Zn) because it forms high-molecular-weight, uncharged complexes [40]. In deionised water infusions, which lack competing ions, Cu^{2+} extraction from the tea matrix was maximised, with green tea infusions showing a greater increase than black tea infusions. However, the intrinsic Cu^{2+} levels of the source teas are unknown. As with other elements, Cu^{2+} concentrations in tea infusions are influenced by the tea's geographical origin, inherent copper content, and infusion duration [41]. Although brewing temperature and time were varied in the present study, no kinetic profiling of ion release was performed; time-resolved extraction studies would aid in distinguishing rapidly leached ions from those requiring structural breakdown of the leaf matrix.

The contribution of water composition to final mineral concentrations is evident across conditions; however, the absence of full mass balance data, specifically, comprehensive elemental profiling of dry

tea leaves, brewing water, and any additives, limits definitive source attribution for individual ions. Future studies should incorporate such profiling to enable more robust source differentiation and mechanistic modelling. Analytical constraints were also encountered for Mn^{2+}, for which concentrations exceeded the calibrated detection range and required separate calibration, highlighting the importance of accounting for matrix-specific interferences and instrument sensitivity in future assay design.

Taken together, the mineral data suggest that water mineral content is an important determinant of post-infusion elemental composition, with deionised water infusions generally maximising leaf-derived ion extraction, tap water infusions representing an intermediate condition, and high-salt water infusions associated with substantial complexation, precipitation, and net removal of both macro- and microelements from solution; these ion dynamics establish the chemical framework within which the physicochemical and biochemical properties of the infusion are subsequently shaped.

Chemical and physical properties

Analyses of the chemical and physical properties of the different tea infusions revealed consistent and reproducible trends across both green and black tea, which can be attributed to the mineral composition of the water used (Fig. 1).

Total Polyphenol Content (TPPC) (Fig. 1, 2a–2b) and **Antioxidant Capacity (AC)** (Fig. 1, 3a–3b) showed broadly similar numerical trends in response to brewing conditions and water type, though, as detailed below, the two measures did not always agree on which differences reached statistical significance. As expected, green tea infusions exhibited numerically higher TPPC than black tea infusions across brewing conditions. At the one condition tested identically for both teas (100°C, 10 min), a two-way ANOVA confirmed this as a significant effect of tea type on TPPC ($p = 0.036$), with no significant tea × water interaction ($p = 0.936$), indicating that green tea's TPPC advantage over black tea held consistently across water types at this condition. For AC, by contrast, the same two-way ANOVA at this condition found no significant effect of tea type ($p = 0.53$), water quality ($p = 0.42$), or their interaction ($p = 0.50$); despite the numerical trends visible in Fig. 1, the apparent AC differences by tea type did not reach statistical significance at this condition. This is primarily attributable to the higher native catechin content of green tea and the limited enzymatic oxidation during its processing, which preserves the phenolic compounds in their monomeric, highly active forms [42]. This aligns with findings from Klepacka et al., who reported higher phenolic levels in green tea infusions (152.12 mg/100mL) compared to black and pu-erh teas (43.38–46.03 mg/100 mL) [27]. Truong et al. further confirm green tea's superior AC relative to oolong and black teas, underscoring the strong correlation between total antioxidant potential and polyphenol content [43]. This difference is largely attributed to variations in manufacturing processes; oxidative processing in oolong, yellow, black, and dark teas significantly reduce polyphenol levels compared to less processed white and green teas [44]. As noted by Truong et al. the total polyphenol content of green tea accounts for 10–15% of dry tea weigh, characterized by a high concentration of monomeric catechins; primarily epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [43, 45]. In contrast, black tea contains about 5% polyphenols of dry tea weight. This reduction is not a loss of antioxidant potential per se, but

rather a transformation; during processing, the catechins in black tea are oxidized by polyphenol oxidases and peroxidases into complex dimers and polymers, such as theaflavins and thearubigins [46, 47]. Consequently, while green tea relies on a higher quantity of catechins for its functional properties, the AC of black tea is driven by these potent, newly formed oxidative derivatives; a distinction with important implications for how brewing conditions differentially affect each tea type

Both TPPC and AC generally increased with higher infusion temperatures and longer extraction times. For instance, in deionised water, green tea infusions TPPC showed a numerical (though not statistically significant) increase from 0.51 mg/L at 75°C to 0.63 mg/L at 100°C, with a corresponding increase in AC from 2.41 to 2.80 gTE/L. Similarly, extending black tea infusion from 5 to 10 minutes at 100°C in deionised water increased TPPC from 0.47 mg/mL to 0.54 mg/mL ($p < 0.05$); the corresponding increase in AC, from 1.93 to 2.19 gTE/L, was numerical but did not reach statistical significance. These findings align with Palanivel et al. (2018), who noted that increased brewing temperature enhances elution and biochemical compound extraction. Vinci et al. similarly reported higher TPPC with longer steep times and at 100°C compared to 80°C, for both green tea and black tea [48]. This corroborates the well-established role of thermal energy in disrupting plant cellular matrices, increasing cell wall permeability, and facilitating the diffusion into the brewing medium [49], which are key to radical-scavenging capacity [8]. The main polyphenols of fresh tea leaves are flavan-3-ols, whose content and structure change depending on technological processes [44]. However, as the identity of these catechin monomers has already been introduced in the preceding paragraph, it is worth noting here that the thermal extraction of these compounds is not indefinitely additive; prolonged exposure to high temperatures can promote epimerisation of EGCG to GCG and hydrolysis of ester catechins, potentially reducing the overall antioxidant profile of the infusion at extreme conditions [49, 50]. The observed increases in TPPC and AC with temperature in the present study therefore likely reflect conditions where extraction gains outweigh thermal degradation losses, particularly within the 75–100°C range examined.

A notable trend across both tea types was the general reduction of TPPC and AC in mineralised waters (TW and HS) compared to deionised water, indicating that increased water hardness negatively impacted polyphenol extraction or solubility. This reduction reached statistical significance in black tea infusions across all conditions tested, whereas the corresponding trend in green tea infusions, though numerically consistent, did not reach significance. For AC, the reduction in mineralised waters was not statistically significant for either tea type at the conditions directly compared, with the exception of black tea in high-salt water at 5 minutes. This pattern was consistent regardless of brewing temperature or steeping time, suggesting that water mineral content exerts an overriding chemical influence on polyphenol recovery [51]. Numerous studies corroborate this, by focusing on the behaviour of catechins, the primary polyphenolic constituent in tea. These studies report lower total catechin content in green tea prepared with tap or mineral water than with deionised or distilled water [5, 10, 52]. For instance, Xu et al. found that catechin content significantly decreased when Ca^{2+} concentration exceeded 40 mg/L [53]. This was attributed to the binding of Ca^{2+} to catechins. This complexation reduces the free catechin pool available for extraction by forming insoluble or poorly soluble Ca^{2+}–polyphenol complexes,

effectively sequestering phenolic compounds within the leaf matrix or precipitating them from solution [54, 55]. While the present study measured TPPC rather than individual catechins, the reductions observed in mineralised waters are likely driven by these ion–polyphenol interactions, with the magnitude of reduction scaling broadly with mineral content: most pronounced in high-salt water infusions, intermediate in tap water infusions, and least in deionised water infusions. The impact of water chemistry on phenolic recovery involves a complex interplay of extraction inhibition and chemical transformation [8, 56]. Vinci et al. [48] reported the highest TPPC for both green tea and black tea infusions when using natural mineral water, suggesting that specific mineral profiles may occasionally favour extraction; the broader literature [21, 52, 56, 57] and the current findings consistently point toward a general inhibitory effect of high mineral content on polyphenol recovery and AC.

The relative reduction in TPPC and AC in mineralised waters (TW and HS) is primarily attributed to their composition. They contain substantial concentrations of dissolved mineral salts, principally Ca^{2+} and Mg^{2+} and hydrogen carbonate ions HCO_3^-. These constituents act through two distinct but interrelated mechanisms: direct chemical interaction with polyphenolic compounds, and inhibition of the extraction process itself. With respect to direct chemical interactions, the B-rings of catechins represent a key structural site for complexation with Ca^{2+} and Mg^{2+} during brewing, with carboxyl and hydroxyl groups on co-extracted organic acids additionally forming five-membered chelating rings with these divalent cations [15]. While Li et al. examined these interactions primarily in the context of scale inhibition, the same Ca^{2+}/Mg^{2+}–catechin B-ring complexation mechanism directly explains the reduction in free-radical-scavenging hydroxyl groups observed in the present study, since the vicinal dihydroxyl group on the B-ring and the galloyl group at the 3-position of the C-ring are the precise structural features responsible for radical scavenging capacity [50, 58]. Complexation of these sites by divalent cations therefore simultaneously reduces both the availability of free catechins in solution and the antioxidant efficacy of those that are extracted, producing a compounded depression in both TPPC and AC [8, 56]. Supporting this, Franks et al. [5] demonstrated that increasing ionic strength reduces catechin stability and extractability, and Li et al. [15] quantified the scale of this ionic sequestration effect, measuring a reduction in free Ca^{2+} from 100.0 to 60.0 mg/L under brewing conditions, consistent with substantial complexation of both Ca^{2+} and polyphenolic hydroxyl groups into stable insoluble complexes.

A notable exception to the general trend of mineralised water suppressing antioxidant capacity was observed for black tea infused in high-salt water at 100°C for 10 minutes, which yielded the highest AC among all black tea infusion conditions (2.43 gTE/L; Fig. 1, 3b). This result is not directly proportionate to the corresponding TPPC measurement and therefore cannot be explained by polyphenol concentration alone. One plausible explanation lies in the specific ionic composition of high-salt water. Klepacka et al. reported significant positive correlations between mineral ion concentrations and antioxidant potential in tea infusions, with Mg^{2+} ($r = 0.7472$), K^+ ($r = 0.5158$), and Mn^{2+} ($r = 0.4587$), all positively associated with AC, and with Mg^{2+} and Mn^{2+} contents showing significant correlations with polyphenol levels, an effect largely determined by the type of phenolic compounds present [27]. The researchers also indicated significant correlations between Mg^{2+} and Mn^{2+} contents and polyphenol

levels, largely determined by the type of phenolic compounds present in tea. The antioxidant contribution of manganese is well-established through its role as the essential catalytic cofactor in manganese superoxide dismutase (MnSOD/SOD2), a principal mitochondrial antioxidant enzyme responsible for neutralising superoxide anion radicals, with documented protective roles across a broad range of oxidative-stress-related diseases [59]. The mechanisms by which Mg^{2+} and K^{+} enhance antioxidative potential in food matrices remain less well characterised. It is therefore possible that under the specific conditions of HS brewing at 100°C for 10 minutes, the net ionic enhancement of antioxidant capacity outweighed the inhibitory effect of Ca^{2+}/Mg^{2+} complexation on catechin-type scavenging, producing an anomalously high AC reading despite moderate TPPC.

The theaflavin-rich composition of black tea may additionally confer an advantage under these conditions, as theaflavins, particularly TF3, TF2A, and TF2B, have been shown to exhibit DPPH radical-scavenging activity exceeding that of epigallocatechin gallate (EGCG) under certain assay conditions (Koch 2020), and studies using low-density lipoprotein oxidation models have ranked their activity as TF3 > EGCG > TF2A in terms of oxidative stress inhibition [60] .That green tea infusions yielded significantly higher TPPC than black tea infusions at the one condition tested identically for both tea's (100°C, 10 min; $p = 0.036$), and numerically higher TPPC under the remaining conditions, is therefore attributable not to the absence of antioxidant activity in black tea infusions, but is more plausibly explained by the lower total extractable polyphenol mass resulting from oxidative polymerisation during processing, along with the differential aqueous solubility of high-molecular-weight thearubigins — although polymer content and thearubigin solubility were not directly measured in the present study and this explanation remains a proposed mechanism rather than a tested finding. By contrast, the corresponding AC difference between green and black tea did not reach statistical significance at this condition ($p = 0.53$), despite the numerical trends visible in Fig. 1, suggesting that theaflavins may substantially compensate for black tea's lower polyphenol mass in terms of measurable AC specifically, even where the underlying TPPC gap is real. Theaflavins may additionally be less susceptible to the same divalent cation complexation pathways that suppress catechin-type scavenging, potentially preserving radical-scavenging capacity even in mineralised water. The interaction between water mineral composition, tea type, and antioxidant compound class therefore merits further investigation, particularly in the context of kombucha production where mineralised water is commonly used.

The differences in TPPC between green tea infusions and black tea infusions, significant at the shared 100°C/10 min condition ($p = 0.036$), reflect the fundamental biochemical divergence introduced by the oxidation process; the corresponding AC difference did not reach significance at this condition. Green tea retains the full complement of monomeric catechins, principally epigallocatechin gallate, epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), which are potent free radical scavengers and the primary contributors to antioxidant capacity in unoxidised tea [52]. During partial oxidation, as in oolong tea production, catechins are partially oxidised by endogenous polyphenol oxidases and peroxidases to produce dimeric structures including theasinensins and theaflavins, as well as other higher molecular weight polyphenols [47]. In fully oxidised black tea, this transformation is far

more extensive: the majority of monomeric catechins are converted into theaflavins and thearubigins, fundamentally altering the polyphenolic profile of the infusion [27, 46].

The extractability of individual catechin fractions is further modulated by solvent polarity. Danrong et al. demonstrated that non-ester catechins predominate in highly polar aqueous solvents such as deionised water, while ester catechins are more abundant in less polar environments, consistent with the differential extraction profiles observed across water types in the present study [56]. Beyond polarity, catechin stability depends critically on brewing medium pH, with alkaline conditions accelerating autoxidation and epimerisation as discussed in the context of bicarbonate-rich mineralised waters [50, 61]. This stability is particularly relevant when comparing the results of deionised water infusions to those of mineralized waters; the latter often possess higher alkalinity due to bicarbonate ions, which can elevate the pH and accelerate the degradation of phenolic structures. Overall, the observed trends in TPPC and AC underscore the profound influence of matrix conditions, specifically mineral load, solvent polarity, and thermal treatment, on the extraction efficiency and functional quality of tea infusions. These findings have direct implications for kombucha-style fermentation applications, where the polyphenol composition and antioxidant capacity of the tea infusion collectively determine the substrate available to the fermenting microorganisms and ultimately shape the fermentation kinetics and bioactive compound profile of the final product.

The ionic and complexation mechanisms that suppressed polyphenol extraction in mineralised waters were also reflected in the turbidity profiles of the infusions (Fig. 1: 1a). Quantitatively, infusions prepared with tap water at 75°C registered a turbidity of 36.1 EBC, which significantly increased to 58.75 EBC when the brewing temperature was raised to 100°C ($p < 0.05$). This effect was exacerbated in high-salt water infusions, where corresponding turbidity values reached 43.1 EBC at 75°C and 85.25 EBC at 100°C, also a significant increase ($p < 0.05$). These observations are consistent with prior research by Xu et al. who reported a sharp increase in green tea infusion turbidity with Ca^{2+} concentrations ranging from 10 to 40 mg/L, followed by a plateau [53].

Black tea infusions exhibited higher turbidity than green tea infusions in tap and high-salt water at the matching 100°C/10 min condition (Fig. 1: 1b); however, as green tea and black tea were analysed in separate statistical models, this difference has not been formally tested for significance. This is likely due to their elevated content of theaflavins and thearubigins which are capable of forming stable, complexed structures [5, 8, 62]. In addition, calcium and magnesium have been identified as the principal elements involved in the formation of tea cream and scum [21, 53, 55], with the former being a visible precipitate that develops as tea cools. This phenomenon is attributed to complexation reactions between caffeine and polyphenolic compounds such as theaflavins and thearubigins. While the latter is defined as surface film composed of calcium, hydrogen carbonates and organic matter, it appears only in infusions prepared with hard water and is produced by the oxidation of organic compounds induced to the presence of calcium carbonate [21].

Notably, black tea infusions prepared with deionised water numerically exhibited the lowest turbidity among the conditions tested (10.0–11.1 EBC), though this comparison, like other cross-tea-type differences discussed above, was not formally tested for statistical significance (see Methods). This finding is consistent with fundamental differences in the colloidal properties of green and black tea particles. Lin et al. demonstrated that green tea turbidity arises from relatively large colloidal aggregates (> 500 nm) with low absolute zeta potentials, conditions that promote flocculation and coalescence into dense, light-scattering clusters. Black tea infusions, by contrast, are characterised by smaller particles (< 200 nm) with higher absolute zeta potentials, conferring stronger electrostatic repulsion and enhanced colloidal stability that favours stable coacervate-like dispersions over extensive aggregation [62]. The turbidity trends observed in the present study are consistent with this colloidal behaviour, with mineral content providing the ionic bridging necessary to overcome electrostatic repulsion and promote aggregation in both tea types, albeit to a greater extent in black tea

Nitrogen-containing compounds, including proteins and free amino acids, are essential components of the tea substrate that support the metabolic activity of the symbiotic culture of bacteria and yeast during fermentation. As black tea is considered among the finest substrates for kombucha preparation, the specific composition of the infusion, particularly its nitrogenous fraction, plays a decisive role in determining the final quality and composition of the fermented beverage [42]. Consequently, even minor alterations in extraction behaviour caused by water mineral content or temperature can significantly influence the availability of these substrates for downstream fermentation. **The Free Amino Nitrogen Content (FAN)** fraction did not exhibit statistically significant variation in response to infusion conditions. Concentrations ranged narrowly between 4.3 and 5.5 mg/L of amino nitrogen (Fig. 1: 4a, 4b). Generally, black tea infusions released slightly higher levels of FAN than green tea infusions, particularly during longer infusion times. In black tea infusions, FAN followed a slight upward trend as water mineral content and duration increased. Conversely, green tea infusions values remained stable across water types or decreased slightly at higher salt concentrations, suggesting a possible interaction between green tea amino acids and high mineral loads that limits their dissolution into the aqueous phase. While most of the literature reports higher FAN or total amino acid content in green tea infusions via liquid chromatography of leaf extracts [22, 63], the present study employed the ninhydrin colorimetric method to quantify the soluble alpha-amino nitrogen liberated into the infusion. This distinction is critical: whereas HPLC provides a profile of total potential amino acids in the leaf, the ninhydrin method quantifies the nitrogen fraction directly available for fermentation, a parameter of higher practical relevance for assessing substrate quality.

Total Protein Content (TPC) demonstrated more dynamic behaviour than FAN. At the one condition tested identically for both tea's (100°C, 10 min), a two-way ANOVA confirmed a significant effect of tea type on TPC ($p = 0.0026$), a significant tea × water interaction ($p = 0.023$), and no significant main effect of water quality alone ($p = 0.922$) indicating that the difference observed in TPC between green and black tea was not consistent across water types, but instead varied depending on which water was used. The largest difference was observed in deionised water infusions, where black tea yielded approximately 152% more protein than green tea (315.6 vs. 125.0 mg/L), and considerably smaller in tap water (252.0

vs. 212.8 mg/L) and high-salt water (239.8 vs. 213.7 mg/L). This phenomenon can be interpreted through the lens of the manufacturing process described by Chen et al. (2020): although total soluble protein within the leaf may decrease during black tea production due to enzymatic degradation, the simultaneous disruption of cellular compartments, specifically the activation of proteases during withering and rolling, likely alters the physical state of proteins, enhancing their extractability. Within green tea infusions, where only brewing temperature was varied, a marked increase in TPC was observed with rising temperature, particularly in mineralised water for instance, TPC in tap water infusions increased from 157.6 to 212.8 mg/L between 75°C and 100°C, suggesting that higher thermal energy overcomes structural barriers in the unoxidized green tea leaf matrix. Within black tea infusions, where only steeping duration was varied, extending infusion time to 10 minutes increased TPC, with deionised water infusions concentrations rising from 278.9 to 315.6 mg/L.

The divergent TPC responses to water mineral content between green tea and black tea infusions reflect fundamental differences in the colloidal stability of their respective infusions. In green tea infusions, both protein and FAN extraction were enhanced with increasing mineral content. This is consistent with the relatively weak binding affinity of non-oxidised, non-galloylated catechins for protein [64]; because green tea catechins form only loose, readily reversible associations with protein chains, divalent cations such as Ca^{2+} and Mg^{2+} preferentially form stable complexes with the ortho-dihydroxyl groups of catechins [15] rather than bridging protein-polyphenol aggregates, leaving the bulk of nitrogenous compounds in solution. This interpretation is further supported by the physical characteristics of green tea cream: Lin et al. demonstrate that green tea cream particles, though larger in diameter, are structurally unstable and easily disrupted. Even where mineral content promotes transient protein-polyphenol associations, the colloidal instability of green tea cream means these complexes are unlikely to persist as precipitate; bound nitrogenous fractions may instead be released back into solution, contributing to the observed increase in measurable TPC and FAN [62].

In black tea, the inverse trend reflects the high creaming affinity of oxidised, polymerised polyphenols. As discussed above, oxidation of catechins produces theaflavins and thearubigins whose greater structural rigidity and stronger protein-binding interactions confer substantially higher colloidal stability than the non-galloylated monomers dominant in green tea. Lin et al. show that theaflavins and thearubigins form cream particles that are significantly more stable and resistant to disruption than their green tea counterparts [62]. In high mineral black tea infusions, divalent cations such as Ca^{2+} and Mg^{2+} act as ionic bridges between these robust protein-polyphenol complexes [53, 65], promoting flocculation and driving the precipitation of nitrogenous compounds out of the liquor. The result is a measurable reduction in both TPC and FAN in the final brew, as soluble protein and free amino nitrogen are progressively incorporated into the cream sediment rather than remaining in solution.

The pH of the infusions varied systematically with both water type and tea matrix (Fig. 1: 3a, 3b). Notably, the direction of pH changes upon infusion differed depending on the starting water chemistry: deionised water (initial pH 5.3) showed a slight increase in pH following infusion with green tea (+ 0.30 at both brewing temperatures), whereas tap water (initial pH 7.49) and high-salt water (initial pH 7.75)

both showed a decrease in pH after infusion. This pattern is consistent with the strong negative correlation between initial water pH and the extent of pH change upon brewing reported by Xu, Hu, et al. ($r = -0.97$, $p < 0.01$) [12], and reflects the contrasting buffering capacity across water types: in deionised water, the absence of buffering ions leaves the system unable to resist even minor pH shifts driven by leaf extraction, while in tap water and high-salt water, the higher initial pH is drawn downward by extracted organic acids, which consume bicarbonate and carbonate alkalinity by donating H^+ and complexing Ca^{2+} and Mg^{2+} [12, 15].

Across water types, deionised water-based infusions exhibited significantly lower absolute pH values than those prepared with tap water or high-salt water, with deionised water-brewed green tea reaching approximately pH 5.6 and deionised water-brewed black tea approximately pH 4.8, whereas tap water and high-salt water infusions remained near neutral (pH 7) regardless of tea type. At the one condition tested identically for both tea's (100°C, 10 min), a two-way ANOVA confirmed significantly lower pH in black tea infusions relative to green tea infusions ($p < 0.0001$), with a significant tea × water interaction ($p < 0.0001$): the difference between the two tea's was far larger in deionised water (5.57 vs. 4.83, a difference of 0.74 pH units) than in tap water (7.08 vs. 6.83, 0.25 units) or high-salt water (7.12 vs. 6.99, 0.13 units).

This pattern is consistent with the greater abundance of acidic oxidation products, theaflavins, thearubigins, and associated gallic acid, generated during the oxidative processing of black tea, which contribute a higher net acid load to the infusion, an effect that is most apparent when unmasked by the absence of buffering minerals in deionised water; indeed, black tea has been reported to exhibit a significantly lower baseline pH than green tea under comparable brewing condition [66]. This mirrors findings reported by Xu, Hu, et al. in their analysis of Chinese teas brewed with different water types [12]. The more acidic environment observed in deionised water-brewed teas may further contribute to enhanced extractability of trace metals and polyphenols, as has been experimentally verified in studies comparing extractions at pH 2 and pH 6 [23], which may partly explain the higher TPPC values observed under deionised water conditions reported above. These findings underscore the complex interplay between water chemistry, temperature, and matrix composition in determining the physicochemical environment of tea infusions.

The **CIELAB** colour space analysis reveals distinct and systematic changes in tea infusion appearance as a function of water type, temperature, and duration. Parameters L* (lightness), a* (green-red), and b* (blue-yellow) offer quantifiable insights into the visual transformation of green and black teas during infusion.

L* values were highest in green tea infusions prepared with deionised water, indicating clearer and more transparent infusions (Fig. 2, L* 98.6□–98.1□). Increasing mineral content significantly reduced L* (Duncan's MRT, $p < 0.05$), with green tea infused in high-salt water at 100°C reaching as low as L* 82.5□, significantly lower than both deionised water (98.1□) and tap water (88.3□) at the same temperature. This reduction is consistent with enhanced turbidity and formation of light-scattering complexes due to

metal-polyphenol and protein-polyphenol interactions [53, 64]. The release of water-insoluble chlorophylls into the infusion may further contribute to turbidity, as these compounds can form emulsion-like suspensions that increase light scattering even under hot infusion conditions [67]. In black tea infusions, inherently lower L* values (51.0-55.4) were observed, particularly under high mineral content, consistent with increased pigment extraction and precipitation phenomena [68]. The brightness is generally ascribed to the theaflavin contents, whereas the colour is related to the thearubigin contents in black tea [34].

The a* values, indicative of green to red shifts, remained negative in green teas brewed with deionised water and tap water (Fig. 2, a* -1.6□/□ to -4.5□), reflecting the preservation of native green pigments due to minimal enzymatic oxidation during green tea processing; at 75°C, deionised water (-1.6□) was significantly less negative than both tap water (-4.3□) and high-salt water (-4.2□) (Duncan's MRT, $p < 0.05$). This chromatic profile is predominantly governed by total chlorophyll content, with the ratio of chlorophyll a (blue-green) to chlorophyll b (yellow-green) playing a central role. Among flavonols, quercetin shows the strongest correlation with infusion greenness, while kaempferol and myricetin contribute less [67]. Catechins, despite their high concentration, are colourless and do not influence hue. Excessive chlorophyll release may cause turbidity [67]. The absence of oxidised polyphenolic compounds, such as theaflavins and thearubigins, which are responsible for the red-brown hues in oxidised teas, further contributes to the retention of the green tea's unoxidized pigment profile [30]. However, green tea brewed in high-salt water at 100°C resulted in a significant shift into the positive a* axis (+ 1.2□), significantly higher than both deionised water (-2.1□) and tap water (-2.0□) at the same temperature ($p = 0.044$), signalling degradation of chlorophyll pigments and the concurrent development of oxidation-driven red-brown chromophores. The higher mineral content and elevated pH of high-salt water have been shown to accelerate catechin degradation and isomerisation, increasing susceptibility to oxidative polymerisation and colour darkening (Cao et al. 2021), with Ca^{2+}, Fe^{3+}, and Cu^{2+} in particular known to accelerate polyphenol oxidation reactions in tea infusions [69].

In black tea infusions, a* values were significantly higher across all water types compared to deionised water (Duncan's MRT, $p < 0.05$ within each condition), reaching up to + 23.1□ in high-salt water-brewed samples after 10 minutes, significantly higher than tap water (19.6□) and deionised water (8.5□) at the same condition; reflecting the robust formation of reddish-brown chromophores, primarily theaflavins and thearubigins, arising from the extensive enzymatic oxidation of catechins during fermentation [34, 47, 70]. At the condition tested identically for both tea's (100°C, 10 min), a two-way ANOVA confirmed significantly higher a* in black tea than green tea ($p < 0.0001$), with a significant tea × water interaction ($p < 0.0001$), indicating that mineralised water intensified red-brown coloration far more strongly in black tea than in green tea. Elevated mineral concentrations and prolonged infusion times further exacerbated this effect [71]. Higher brewing temperatures significantly enhanced elution of biochemical compounds from black tea leaves, with theaflavin and catechin solubilisation reaching peak levels at 95°C after 15 minutes; theaflavins formed early during oxidation subsequently convert to thearubigins, which are directly linked to the colour intensity of black tea infusions [71]. Furthermore, Minamoto et al. demonstrated that higher water hardness decreased the yields of theaflavin and thearubigin while

conversely increasing the yield of highly polymerised substances, resulting in a darker-coloured brew [72].

The b* values followed a clear trend across both tea types. Green tea brewed in deionised water remained pale yellow (b* 7.6□–10.8□), significantly lower than tap water and high-salt water infusions, which exhibited intensified yellow-orange hues (b* 29.3□/□–43.3□) (Duncan's MRT, $p < 0.05$ within each condition), consistent with increased catechin oxidation and polyphenol complexation under mineralised conditions [72, 73]. Black tea infusions exhibited markedly higher b* values across all conditions; notably, the most intense yellowness (b* > 70□) occurred in deionised water samples, significantly higher than tap water (68.2□) and high-salt water (64.5□) at the matching 100°C/10 min condition — the reverse of the pattern seen in green tea. At this shared condition, a two-way ANOVA confirmed significantly higher b* in black tea than green tea overall ($p < 0.0001$), with a significant tea × water interaction ($p < 0.0001$) reflecting this divergent, tea-type-dependent response to mineral content. This apparent inversion likely reflects the fact that in deionised water, theaflavins, which contribute orange-yellow colour, remain in solution rather than being incorporated into the darker, more polymerised complexes that form preferentially with high mineral content [72]. In tap water and high-salt water, the formation of these highly polymerised dark brown pigments offsets and suppresses the yellow-orange contribution of theaflavins, resulting in lower b* despite higher overall pigment extraction. Collectively, these colourimetric shifts reflect the underlying chemistry of polyphenol oxidation, complexation, and precipitation as a function of water mineral content and tea matrix, parameters of direct relevance to the sensory and visual quality of tea-based fermentation substrates.

Conclusions

The present study demonstrates that even subtle variations in mineral composition, buffering capacity, and tea-matrix chemistry fundamentally redefine the physicochemical environment of the sweetened tea base. While deionised water infusions enhance the extraction and stability of polyphenols by preventing their precipitation, tap water and high-salt water infusions trigger complexation. This results in increased turbidity and a measurable loss of dissolved polyphenol content. Furthermore, intensifying the infusion parameters by increasing the temperature from 75°C to 100°C in green tea and extending the infusion time from 5 to 10 minutes in black tea resulted in significantly elevated turbidity levels. These observations align with the established role of calcium and magnesium in promoting polyphenol-mineral complexation and precipitation during infusion.

These matrix-dependent shifts, affecting pH, nutrient availability, polyphenol composition, and colour characteristics, are expected to carry meaningful consequences for microbial performance during kombucha fermentation. Given that kombucha represents a system in which polyphenol-derived antimicrobial activity and nutrient availability jointly constrain microbial growth, such variations in the starting substrate are likely to influence fermentation dynamics, acid and metabolite formation, and final product quality.

Declarations

Conflicts of Interest

The authors declare no conflict of interest.

Compliance with ethics requirements

All authors were compliant and followed the ethical guidelines, according to the requirements of European Food Research and Technology.

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Author Contribution

CRediT authorship contribution statement GA: Conceptualisation, Methodology, Formal analysis, Investigation, Visualisation, Writing - review & editing. ML: Conceptualisation; Methodology, Formal analysis, Investigation, Visualisation, Writing - review & editing. JL: Methodology, Investigation, Visualisation. SR: Methodology, Investigation. BG: Writing - review & editing, Supervision. MS: Conceptualisation, Formal analysis, Writing - Original Draft, review, Visualisation, Validation, Funding acquisition, Supervision.

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Data Availability

All data supporting the findings of this study are available within the paper.

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Tables

Tables are available in the Supplementary Files section.

Figures

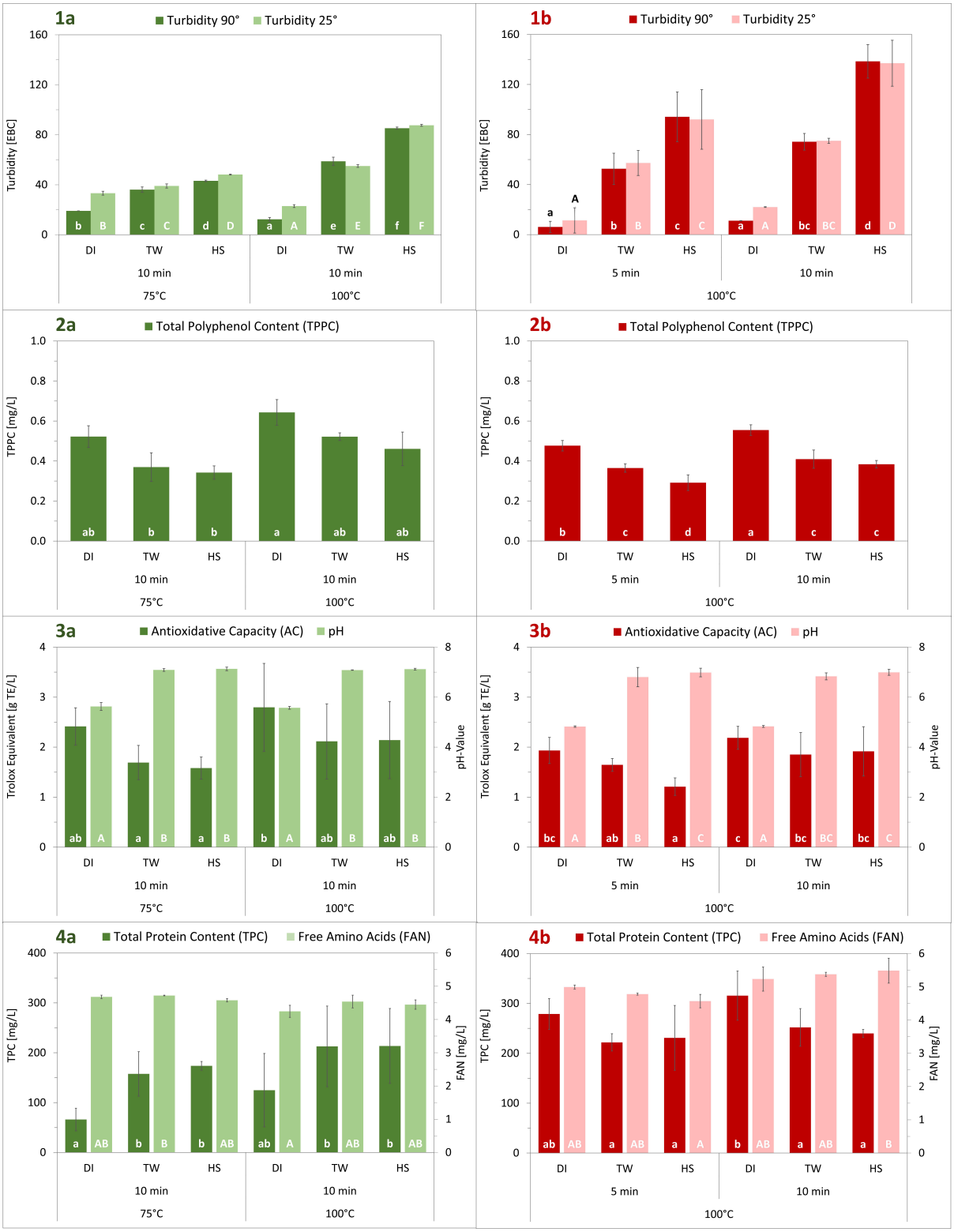


Figure 1

Characteristics of sweetened green (a) and black (b) tea after infusion under different time-temperature regimes and using three different degrees of water mineral content (deionised water (DI), tap water (TW), and high-salt water (HS)). Illustrated are (1) turbidity at 90° and 25°, (2) total polyphenol content, (3) antioxidative capacity expressed in Trolox equivalents and pH, and (4) Total protein content and free amino nitrogen. Data are presented as mean ± SD from four independent replicates. Within each

histogram, different letters indicate significantly different mean values, as determined by one-way ANOVA followed by Duncan's post-hoc test ($p < 0.05$)

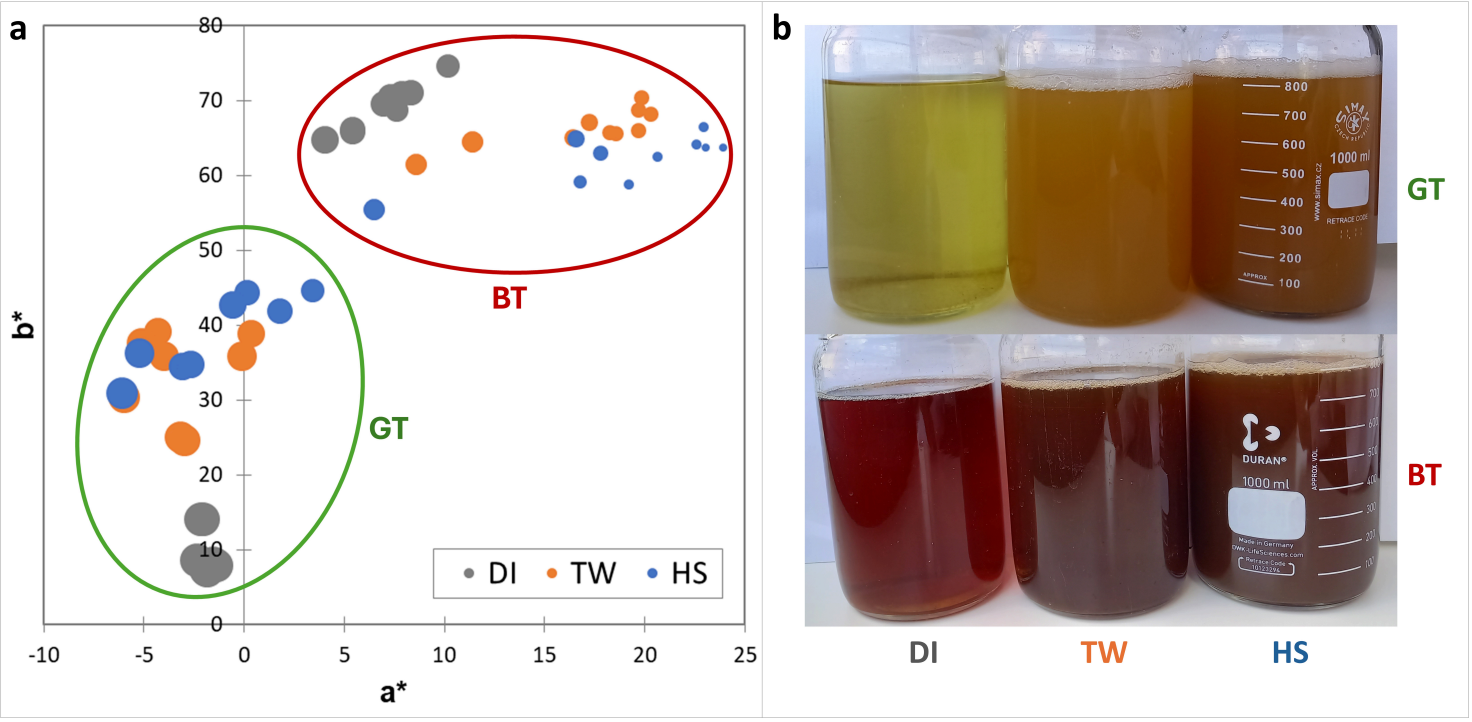


Figure 2

a: Scatter plot of Lab measurements illustrating colour differences in sweetened green tea (GT) and black tea (BT) infusions prepared under different time-temperature conditions and using three water types with varying mineral content (DI: deionised water, TW: tap water, HS: high-salinity water). L is visualised through dot size (larger dot = higher L*, smaller dot = lower L*), while a* indicates chromaticity along the red-green axis (positive = red, negative = green) and b* along the yellow-blue axis (positive = yellow, negative = blue). The largest and smallest dots correspond to L* values of 98.83 and 48.41, respectively. Data represent mean values from four independent replicates. b: Representative images of the tea matrices infused for 10 min at 100 °C.

Supplementary Files

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- Table1and2.xlsx
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