



## Optimizing Extraction Parameters to Minimize Tannin Content in Uva Black Tea Infusions

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### Abstract

Uva black tea contains more tannins than other tea types, contributing to its rich color and astringency; however, excessive tannin levels may pose potential health risks, prompting efforts to reduce its content. Green tea studies suggest that lower temperatures, shorter infusion times, and metal ions suppress tannin leaching. Therefore, this study aimed to evaluate the impact of different extraction temperatures, time, water hardness, and calcium (Ca) and magnesium (Mg) ion content on tannin leaching levels in Uva black tea. Because tannins are high-molecular-weight polyphenolic compounds, measuring both total polyphenol and tannin concentrations was necessary to evaluate how water composition influences tannin removal. Polyphenol and tannin content were quantified spectrophotometrically. The results indicated that tannin concentration increased with higher extraction temperature and time. Hard water produced a darker color compared to soft water, though polyphenol and tannin levels showed no significant difference. Filtration with a coffee filter did not affect color, but centrifugation lightened the supernatant. Both treatments tended to reduce polyphenol and tannin concentrations. Ca and Mg are abundant in hard water. Therefore, in this study these ions were added individually and together to assess their effects on tannin concentration. The Ca-added group showed a color similar to hard water, while the Mg-only group resembled soft water. Varying Ca concentration did not raise pH, indicating that color change was not due to pH. These findings suggest that Ca contributes to color increase. To reduce tannins in black tea, using hard water to bind Ca with tannins and removing them through filtration is effective. Using a fine-mesh filter may further enhance the tannin reduction effect.

### Introduction

Uva black tea is a type of black tea primarily produced in Sri Lanka and enjoyed worldwide alongside

Assam and Darjeeling teas. Black tea has a higher concentration of tannins than other tea types, contributing to its vibrant color and characteristic bitter taste. The tannin amount significantly affects the quality of black

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tea (Kozaki et al., 2020). However, excessive consumption of tannin-rich foods has been associated with adverse health effects such as gastrointestinal discomfort, liver toxicity, and nutrient malabsorption (Arul et al., 2023; Nghia et al., 2023). Previous studies have demonstrated the effects of excess tannins on humans. No substantial changes were observed in the liver and kidney function, or blood components of human subjects when they were exposed to 480 mg/day or 960 mg/day of tannins for 6 months or 5 weeks, respectively (Maugeri et al., 2022). However, another study demonstrated decreased iron concentration in plasma when exposed to higher doses for 4 weeks (Maugeri et al., 2022). The tannin intake from regular tea consumption is considered relatively low. However, continuous intake of relatively large amounts of tannin may have adverse health effects. Therefore, optimizing extraction conditions to reduce tannin content while preserving desirable flavor and nutritional components is of growing interest.

Recent studies have highlighted that extraction parameters such as temperature and time and water composition affect the release of polyphenols and tannins from tea leaves. For instance, high water temperatures ( $\geq 90^{\circ}\text{C}$ ) are more effective for phenol extraction from black tea compared to room temperature (Venditti et al., 2010). In addition, Zeng et al. (2017) demonstrated that higher extraction temperatures significantly increased the concentration of tea polyphenols and catechins. At  $100^{\circ}\text{C}$ , polyphenol content was higher compared to that obtained with extractions conducted at  $25^{\circ}\text{C}$  or  $4^{\circ}\text{C}$ , indicating that elevated temperatures enhance solubility and diffusion of these compounds. Conversely, lower temperatures and shorter infusion times can limit tannin release. Earlier studies of green tea by Ikeda et al. (1972) demonstrated that lower extraction temperatures and shorter times result in less tannins being eluted, a trend recently confirmed in black tea by Yücel and Kaya (2024). Based on these findings, it is reasonable to infer that tannin elution also increases with temperature, potentially intensifying astringency in black tea.

Tannins also react with alkaline earth metals to form precipitates. For example, when calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) was added at 0.5–6% to the water extract of stevia leaves, the tannin concentration decreased from 643 mg/L to 421 mg/L (Polban et al., 2025). Tannins combined with metal ions, such as  $\text{Al}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$ , can reduce metal toxicity and improve biocompatibility; tannin-based adsorbents have recently been used to remove heavy metals and dyes from

wastewater (Zhang et al., 2023). This was further supported by the decrease in tannin concentrations in black tea when extracted in water with varying hardness levels.

Recent studies have confirmed that water hardness significantly affects the flavor, antioxidant activity, and catechin content of tea infusions. For example, Cabrera et al. (2021) demonstrated that high hardness water reduce catechin content in green tea, while Minamoto et al. (2023) showed that the antioxidative activity of the Ishizuchi dark tea infusion was clearly independent of water hardness. Additionally, Nakashima et al. (2022) reported that water hardness influences bitterness in green tea and umami in black tea, as measured by taste sensors.

Previous studies have focused on sencha, or black tea leaves with lower tannin contents (Kubota et al., 1959; Sakamoto et al., 2012), but these are now cited primarily for historical context. While the foundational effects of extraction temperature, time, and water hardness have been explored, few studies have quantitatively examined these parameters in high-tannin cultivars such as Uva.

This study aims to address this gap by investigating the effects of extraction temperature, time, and water hardness, particularly the roles of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions, on the polyphenol and tannin concentrations of Uva black tea. Additionally, the effect of metal ions on tannin reduction was examined by assessing water color and the pH of black tea extracted with hard and soft water. The findings of this study are expected to provide a scientific basis for optimizing extraction conditions to reduce tannin concentrations during the brewing process across different types of tea.

## Materials and methods

### 1. Materials

Ceylon Uva black tea (from Sri Lanka) was purchased at a retail store in Tokyo (TOPVALU: Green Eye Organic & Fair-Trade Ceylon Uva Black Tea, AEON CO., LTD, Japan).

### 2. Effect of extraction temperature and time on polyphenol and tannin levels in Uva black tea

To investigate the effect of extraction temperature on the polyphenol and tannin content of Uva black tea, tea packets (2 g each) were steeped in 150 mL of water at four different temperatures (40, 60, 80, or  $90^{\circ}\text{C}$ ). with one packet per temperature condition. One tea bag was used for each temperature condition and brewed for 3 min, following the preparation instructions provided on

the product packaging. After extraction, the polyphenol and tannin concentrations of the tea infusions were determined.

To examine the effect of extraction time, tea bags (2 g) were steeped in 150 mL of water at the same four temperatures (40, 60, 80, and 90°C) for varying durations of 2, 3, 6, 8, and 10 min. The selected temperature range represents practical brewing conditions commonly applied in both household and industrial tea preparation. Specifically, 40°C represents mild extraction conditions similar to lukewarm or cold infusion, whereas 90°C approximates near-boiling water typically used for hot tea preparation. The five extraction times were selected to capture the dynamic release of polyphenols and tannins during the early, intermediate, and later stages of steeping. Short extraction times (2–3 min) reflect typical consumer brewing practices, while extended extraction periods (6–10 min) allow for the assessment of continued compound release and the potential onset of saturation or plateau effects in the extraction process.

To measure the polyphenol content of black tea, 200 µL of black tea extract was mixed with 800 µL of 1% hydrochloric acid methanol (Wako, Fujifilm Wako Pure Chemicals Corporation, Japan). Subsequently, 125 µL of the mixture was mixed with 250 µL of Folin–Ciocalteu reagent (Nacalai, Nacalai Tesque, Inc., Japan) and allowed to stand for 3 min; 325 µL of 1% sodium carbonate (Wako, Fujifilm Wako Pure Chemicals Corporation, Japan) was added and mixed well. Thereafter, absorbance at 750 nm was determined using a plate reader (Infinite F50, Tecan Japan Co., Ltd., Japan). Gallic acid (Nacalai, Nacalai Tesque, Inc., Japan) was used as the standard substance in the polyphenol measurement and the concentration of polyphenol in the sample solution was calculated using the calibration curve equation:  $y = 0.0027x + 0.0416$ . Polyphenol concentrations are expressed as gallic acid equivalents (GAE) (Kasai et al., 2024).

The tannin content was measured by combining 0.1 mL of black tea extract, 0.3 mL of iron tartrate reagent (0.1% iron sulfate (Cica, Kanto Chemical Co., Inc., Japan), 0.5% potassium sodium tartrate (Wako, Fujifilm Wako Pure Chemical Corporation, Japan) and 0.6 mL of 0.1 M phosphate buffer (pH 7.5) potassium dihydrogen phosphate (Nacalai, Nacalai Tesque, Inc., Japan). Thereafter, the absorbance at 540 nm was determined using the plate reader. Tannic acid (Wako, Fujifilm Wako Pure Chemicals Corporation, Japan) was used as the standard substance in the tannin measurement and the

concentration of tannins in the sample solution was calculated using the calibration curve equation:  $y = 0.1252x + 0.0729$ . Tannin concentrations are expressed as tannic acid equivalents (TAE) (Iwasa & Torii, 1962).

### **3. Effect of water hardness on polyphenol and tannin content in Uva black tea extract**

To examine the effect of water hardness on the polyphenol and tannin contents of Uva black tea, tea bags (2 g) were steeped in 150 mL of either soft or hard water that had been heated to a rolling boil (100°C). The boiling water was immediately used for extraction, and the tea bags were infused for 3 min. Boiling water was employed to reflect typical tea preparation practices and to ensure that comparisons of water hardness were conducted under realistic brewing conditions. The polyphenol and tannin concentrations of the resulting tea extracts were determined as described in Section 2 of the Materials and Methods. Tap water (hardness: 60 mg/L) was used as the soft water source, whereas commercially available mineral water (Evian; hardness: 304 mg/L, Evian, Itoen Itochu Mineral Waters Co., Ltd., Japan) was used as the hard water source.

### **4. Verification of tannin removal effects using water hardness**

Water coloration has been used as a simplified indicator of total polyphenol and tannin content in tea extracts (Uehara, 2017). Following this approach, the present study measured the color intensity of Uva black tea extracts using absorbance at 450 nm. Uva black tea was subjected to hard and soft water treatments. Each treatment was boiled to 100°C and filtered immediately through a coffee filter (TOPVALU, AEON Co., Ltd., Japan) or centrifuged (9896 × g, 5 min, 25°C) (CF16RN, Eppendorf Himac Technologies Co., Ltd., Japan) to assess water color, polyphenol, and tannin content. The filtrate or supernatant color, as well as polyphenol and tannin contents were measured based on absorbance at 450 nm using the plate reader. Water hardness was measured as described in Section 3 of the Materials and methods section.

### **5. Effect of metal ions on Uva black tea water coloration**

To isolate the effects of individual metal ions on tea coloration, a tea packet (2 g) was placed in 150 mL of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , or mixed solutions that was prepared at specified concentrations. These were then boiled to 100°C immediately before extraction and extracted for 3 min. The water color of the extract was assessed by measuring absorbance at 450 nm. The following solutions were

prepared:  $\text{CaCl}_2$  solution (22.08 mg/100 mL: 8.0 mg/100 mL as Ca; Calcium chloride, Wako, Fujifilm Wako Pure Chemicals Corporation, Japan), with the Ca concentration same as that in hard water (8.0 mg/100 mL);  $\text{CaCl}_2$  solution (11.73 mg/100 mL: 4.2 mg/100 mL as Ca);  $\text{MgSO}_4$  solution (12.87 mg/100 mL: 2.6 mg/100 mL as Mg) with the Mg concentration same as that in hard water (2.6 mg/100 mL; Magnesium sulfate, Wako, Fujifilm Wako Pure Chemicals Corporation, Japan);  $\text{MgCl}_2$  solution (10.18 mg/100 mL: 2.6 mg/100 mL as Mg; Magnesium chloride, Nacalai, Nacalai Tesque, Inc., Japan); mixed Ca and Mg solution with the same concentrations as that in hard water ( $\text{CaCl}_2$ : 22.08 mg/100 mL +  $\text{MgSO}_4$ : 12.87 mg/100 mL). Two magnesium salts ( $\text{MgCl}_2$  and  $\text{MgSO}_4$ ) were used to assess whether the counter-ion (chloride vs. sulfate) influences the interaction between  $\text{Mg}^{2+}$  and tea components. In Experiment 5, the objective was to investigate whether calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), or their combination contributed to tannin removal through complexation, as reflected by changes in water coloration. Absorbance at 450 nm was used as a simplified indicator of tannin presence, based on the established relationship between tea color and tannin content. Therefore, detailed quantification of phenolic compounds and tannins was not performed in this experiment.

## 6. Effect of pH on Uva black tea water coloration

To distinguish the effect of pH from that of Ca ions, Uva black tea (2 g) was extracted for 3 min using 150 mL of 1% sodium carbonate (Wako, Fujifilm Wako Pure Chemicals Corporation, Japan) solution that was pre-adjusted to pH 6, 7, 8, or 9 and then boiled to 100 °C immediately before use. The water color of the extract at each pH was measured using absorbance at 450 nm. The pH of different  $\text{CaCl}_2$  solutions (0–22.08 mg/100 mL; 0–8.0 mg/100 mL as Ca) was measured. Sodium carbonate (1%) was prepared by dissolving 1 g of sodium carbonate in water and pre-adjusted to pH 6, 7, 8, or 9 with hydrochloric acid (Hydrochloric acid, Fujifilm Wako Pure Chemicals Corporation, Japan) and adjusted to a volume of 100 mL. The experiment was conducted to investigate whether the removal of tannins in Uva black tea was primarily influenced by the presence of  $\text{Ca}^{2+}$  or by pH changes resulting from increased  $\text{Ca}^{2+}$  concentrations. By independently adjusting the pH using sodium carbonate solutions and comparing the water coloration of tea extracts, the experiment aimed to distinguish the effect of pH from that of  $\text{Ca}^{2+}$  itself. Absorbance at 450 nm was measured to evaluate the

color intensity of the extract as a simplified indicator of tannin behavior. These experiments (corresponding to Figs. 5 and 6) were designed as confirmation tests, and therefore a smaller sample size ( $n = 5$  for Fig. 5 and  $n = 3$  for Fig. 6) are considered sufficient for the intended purpose.

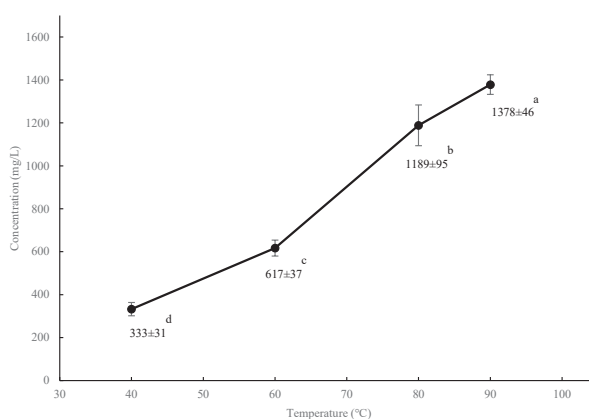
## 7. Statistical analysis

Data were obtained according to Fisher's three principles. For each experimental condition, three independent extractions were performed ( $n = 3/\text{condition}$ ). Differences in means between two groups were assessed using Student's *t*-tests (Microsoft Excel 2019) and differences among three or more groups were assessed using one-way or two-way analysis of variance (ANOVA; Microsoft Excel 2019, Microsoft Corporation, USA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test (R v.4.0.3, Console 64-bit). Statistical significance was set at  $p < 0.05$  for all analyses.

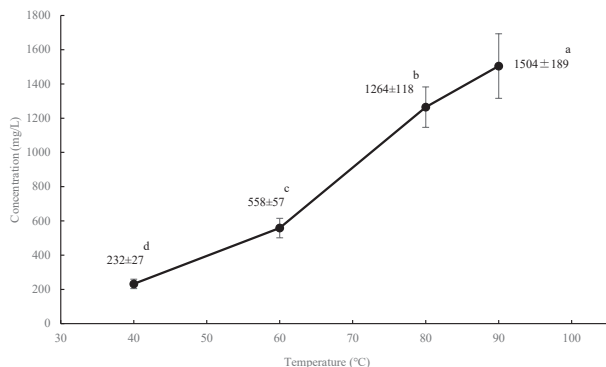
## Results and discussion

### 1. Effect of extraction temperature and time on polyphenols and tannins in Uva black tea

The polyphenol concentration in Uva black tea extracts increased from 333 (GAE) mg/L at 40°C to 1378 (GAE) mg/L at 90°C ( $p < 0.05$ ; Fig. 1). Similarly, the tannin concentration in the extracts increased from 232 mg/L at 40°C to 1504 mg/L at 90°C ( $p < 0.05$ ; Fig. 2). Compared to that at 40°C, polyphenol concentration at 90°C increased by 314%, and tannin concentration increased by 548%.



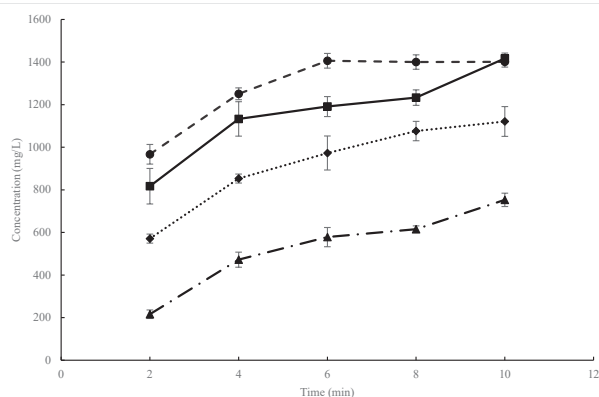
**Fig. 1** Effect of different extraction temperatures on polyphenol concentration in Uva black tea. Letters with different superscripts indicate significant difference ( $p < 0.05$ ). Error bars represent standard deviation;  $n = 9$



**Fig. 2** Effect of different extraction temperatures on tannin concentration in Uva black tea. Letters with different superscripts indicate significant difference ( $p < 0.05$ ). Error bars represent standard deviation;  $n = 9$ .

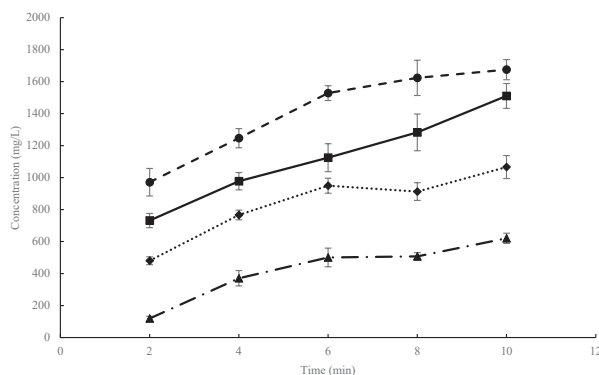
The concentrations of Uva black tea polyphenols and tannins at a constant extraction time (3 min) increased with the increase of extraction temperature. Hajiaghaalipour et al. (2016) have reported similar results; they observed 2,292.2 mg/g of polyphenols in black tea leaves (Assam type) steeped for 5 min in hot water (100°C) and 1,843.0 mg/g in cold water (28°C). Meanwhile, Takaoka et al. (2021) reported increased tannin content in black tea extract (25 mg/100 mL) at 100°C compared to that at 5°C (2.5 mg/100 mL). These results collectively suggest that Uva black tea extracted at high temperatures releases a large amount of tannins and polyphenols.

The effect of different extraction times and temperatures on polyphenol (Fig. 3) and tannin (Fig. 4) concentrations in Uva black tea was also investigated. The polyphenol content increased from 216 (GAE) mg/L in 2 min to 578 (GAE) mg/L in 6 min (168% increase;  $p < 0.05$ ), 615 (GAE) mg/L in 8 min (6.4% increase;  $p > 0.05$ ), and 753 (GAE) mg/L in 10 min (22% increase;  $p < 0.05$ ) at 40°C; from 571 (GAE) mg/L in 2 min to 1076 (GAE) mg/L in 8 min (88% increase;  $p < 0.05$ ), and 1,121 (GAE) mg/L in 10 min (4.2% increase;  $p > 0.05$ ) at 60°C; from 817 (GAE) mg/L in 2 min to 1133 (GAE) mg/L in 4 min (39% increase;  $p < 0.05$ ), 1233 (GAE) mg/L in 8 min (8.8% increase;  $p > 0.05$ ), and 1,417 (GAE) mg/L in 10 min (15% increase;  $p < 0.05$ ) at 80°C; and the concentration increased from 967 (GAE) mg/L at 2 min to 1,406 (GAE) mg/L at 6 min (45% increase;  $p < 0.05$ ) at 90°C. In contrast, polyphenol content decreased to 1,400 (GAE) mg/L at 8 min ( $p > 0.05$ ) and was retained at 1,401 (GAE) mg/L at 10 min ( $p > 0.05$ ). After performing a two-way ANOVA on the data, Tukey's post-hoc test was conducted.



**Fig. 3** Effect of different extraction times and temperatures on polyphenol concentration in Uva black tea extracts. Triangle: 40°C, diamond: 60°C, square: 80°C, circle: 90°C. Error bars represent standard deviation;  $n = 9$ .

Minor differences were observed in the tannin concentrations of Uva black tea. At 40°C, it increased from 119 mg/L at 2 min to 501 mg/L at 6 min (320% increase;  $p < 0.05$ ), increased to 508 mg/L at 8 min (1.4% increase;  $p > 0.05$ ) and increased to 620 mg/L at 10 min (22% increase;  $p < 0.05$ ). At 60°C, the tannin concentration increased from 480 mg/L at 2 min to 949 mg/L at 6 min (98% increase;  $p < 0.05$ ), decreased to 913 mg/L at 8 min (3.8% decrease;  $p > 0.05$ ) and increased to 1066 mg/5 mL at 10 min (17% increase;  $p < 0.05$ ). At 80°C, it increased from 732 mg/L at 2 min to 1511 mg/L at 10 min (106% increase;  $p < 0.05$ ). Similarly, at 90°C, the tannin concentration increased from 971 mg/L at 2 min to 1529 mg/L at 6 min (57% increase;  $p < 0.05$ ), increased to 1624 mg/L at 8 min (6.2% increase;  $p > 0.05$ ), increased to 1675 mg/L at 10 min (3.1% increase;  $p > 0.05$ ). After performing a two-way ANOVA on the data, Tukey's post-hoc test was conducted.



**Fig. 4** Effect of different extraction times and temperatures on tannin concentration in Uva black tea. Triangle: 40°C, diamond: 60°C, square: 80°C, circle: 90°C. Error bars represent standard deviation;  $n = 9$ .

At 90°C, the increase in polyphenol and tannin concentration became more gradual after 6 min. Similarly, the tannin concentration initially increased with extraction time and subsequently plateaued after 6 min for all temperatures, excluding 80°C. Yoneda and Kato (1994) also observed increased tannin concentrations in sencha tea extract at 80°C and 2°C during the early stage of extraction from 0.5 to 4 min, with no significant changes later in the process. Similar results have been reported for green tea extract at 40–95°C; tannin concentrations increased with increasing extraction temperature and time but plateaued after 6 min at 95°C (Ikeda et al., 1972). This indicates that the higher the extraction temperature and longer the extraction time, the greater the amount of polyphenols and tannins are extracted, although the concentration remains constant over time. The amount of tannin in each tea was reported to be 9.4% in Uva black tea (this content was measured in the leaves) (Saito, 1984), 8.39% in green tea (Ikeda et al., 1972), and 4.03% in rooibos tea (Takasugi et al., 2008). The polyphenol concentration in Uva black tea in this study following 10 min extraction was approximately three times higher than in fermented rooibos tea after 10 min (Murakami & Nagasawa, 2021). However, the tannin content in the Uva black tea in this study and in green tea in a previous study (Ikeda et al., 1972) did not significantly change after 6 min, whereas that in the fermented rooibos tea extract continued to increase till 15 min (Murakami & Nagasawa, 2021). In Uva black tea, more polyphenols and tannins were extracted at an earlier stage. Furthermore, Chandini et al. (2011) determined that polyphenol content increased over time when different types of black tea were extracted at 90°C for 10 to 120 min. Although the conditions in this report differ from those in the present study with regard to a maintained temperature of 90°C, both studies showed a similar trend of increasing amounts of polyphenols with the increase of extraction time.

## 2. Effect of water hardness on polyphenol and tannin content in Uva black tea extract

The polyphenol concentration in Uva black tea extract was 1,392 (GAE) mg/L and 1,373 (GAE) mg/L in soft and hard water, respectively, showing a 1.4% decrease with hard water, with no significant differences ( $p > 0.05$ ; Table 1). The tannin concentration was 1237 mg/L and 1203 mg/L in soft and hard water, respectively, a 2.7% decrease, with no significant differences detected ( $p > 0.05$ ; Table 1). Hence, extraction with hard or soft water did not impact the polyphenol or tannin content.

This result may be due to the low Ca concentration in the hard water used, which was insufficient to coordinate effectively with the high tannin content of Uva black tea.

**Table 1** Polyphenol and tannin concentrations in Uva black tea extracted with soft and hard water

|            | Polyphenol<br>Conc. (GAE mg/L) | Tannin<br>Conc. (mg/L) |
|------------|--------------------------------|------------------------|
| Soft water | 1392±47                        | 1237±115               |
| Hard water | 1373±51                        | 1203±58                |
| t-test     | >0.05                          | >0.05                  |

**Remark:** GAE, gallic acid equivalents; Conc., Concentration; ±, standard deviation; n = 9. Extraction was performed for 3 mins using 150 mL of boiling hard or soft water. Data are presented as mean ± SD.

Tannin phenolic hydroxyl groups (-OH) can form hydrogen bonds or coordination complexes with metal cations, such as  $\text{Ca}^{2+}$ , which has a high coordination number (6–8), allowing multiple phenolic groups from one tannin molecule or multiple tannin molecules to coordinate with a single  $\text{Ca}^{2+}$  (Polban et al., 2025). When tannins in stevia leaf were removed with  $\text{Ca}^{2+}$ , 63.7% of tannins were removed by adding 0.5% CaO (Polban et al., 2025). However, the Ca concentration in hard water in this study was very low compared to that reported by Polban et al. (2025), and as the tannin content of the Uva black tea used was high compared to that of other teas (Murakami & Nagasawa, 2021), tannin was thought to be present in excess and could not be removed even when coordinated with  $\text{Ca}^{2+}$ . Furthermore, the Ca concentration in the hard water used in this study was substantially lower than the approximately 1,785 mg/L  $\text{Ca}^{2+}$  achieved by adding 0.5% CaO in the study by Polban et al. (2025). In addition, the tannin content of Uva black tea was considerably higher than that of stevia leaf extract, resulting in an unfavorable tannin-to-Ca molar ratio. These factors likely limited the extent of tannin removal via coordination or precipitation, even though  $\text{Ca}^{2+}$  was present. The reaction between high tannin concentration and low  $\text{Ca}^{2+}$  concentration warrants further research. Nonetheless, these results suggest that the changes in polyphenol and tannin concentrations do not significantly vary with water hardness.

The absorbance of Uva black tea extracted with hard water (1.38) was significantly higher than that of tea extracted with soft water (0.599), representing an increase of approximately 130% ( $p < 0.05$ ; Table 2). For the soft-water extract, absorbance decreased slightly after filtration and centrifugation. Specifically, the absorbance of the untreated extract was 2.6% higher than that of the extract passed through a coffee filter (0.584;  $p > 0.05$ ) and

5.5% higher than that of the centrifuged extract (0.568;  $p < 0.05$ ). Similarly, for the hard-water extract, the absorbance of the untreated sample was 1.5% higher than that of the filtered extract (1.36;  $p > 0.05$ ) and 85% higher than that of the centrifuged extract (0.744;  $p < 0.05$ ). Statistical significance among treatments was determined using analysis of variance (ANOVA) followed by Tukey's post hoc test.

The polyphenol concentration of Uva black tea extracted with soft water was 1,002 mg/L gallic acid equivalents (GAE), which was significantly lower than that of the hard-water extract (1,059 mg/L GAE), representing a 5.7% increase ( $p < 0.05$ ; Table 2). In the soft-water treatment, the polyphenol concentration of the untreated extract was 6.4% higher than that of the filtered sample (938 mg/L GAE;  $p < 0.05$ ) and 6.2% higher than that of the centrifuged sample (940 mg/L GAE;  $p < 0.05$ ). Similarly, in the hard-water treatment, the polyphenol concentration of the untreated extract was 4.8% higher than that of the filtered sample (1,010 mg/L GAE;  $p < 0.05$ ) and 15% higher than that of the centrifuged sample (901 mg/L GAE;  $p < 0.05$ ). All statistical comparisons were conducted using ANOVA followed by Tukey's post hoc test.

The tannin concentration of Uva black tea was 811 mg/L and 901 mg/L in soft and hard water, respectively, showing a 10% decrease with hard water ( $p < 0.05$ ; Table 2). The tannin concentration in untreated soft water extract (811 mg/L) was 6.8% higher after passing through a coffee filter (759 mg/L;  $p < 0.05$ ) and 2.7% higher than after centrifugation (790 mg/L;  $p > 0.05$ ). Meanwhile, the tannin concentration in untreated hard water extract (901 mg/L) was 8.6% higher after being passed through a coffee filter (830 mg/L;  $p < 0.05$ ) and 15% higher after centrifugation (784 mg/L;  $p < 0.05$ ). The data were tested using the Tukey test after performing ANOVA. This significant increase in absorbance in hard water, despite the similar tannin concentrations shown in Table 1, is not due to a higher concentration of tannins but rather owing to a chemical reaction where  $\text{Ca}^{2+}$  form new, darker-colored Ca–tannin complexes.

These results suggest that centrifugation of Uva black tea prepared with hard water reduces color absorbance as well as the concentrations of polyphenols and tannins. This effect may be attributed to the interaction between calcium ions ( $\text{Ca}^{2+}$ ) present in hard water and tannins (Tsuchiya, 2011), which can lead to the formation of suspended complexes that contribute to the darker appearance of the tea infusion. Tannins, which are a subclass of polyphenolic compounds (Okuda & Ito, 2011), may subsequently precipitate during filtration or

centrifugation, thereby reducing both color intensity and the measurable concentrations of polyphenols and tannins in the extract. These findings indicate that when black tea is extracted using hard water, tannin levels can be reduced through physical separation processes such as centrifugation and filtration. However, because centrifugation equipment is generally not available in most household settings, the use of filtration systems with a finer mesh than standard coffee filters may provide a practical alternative for removing these aggregates.

In this study, centrifugation effectively removed complexes formed between metal ions and tannins. Therefore, further research is warranted to investigate the use of finer filtration materials and to explore methods that promote the aggregation of metal–tannin complexes into larger particles that can be more readily separated. Taken together, these results suggest that water hardness does not significantly influence the initial extraction of tannins (Table 1), but rather promotes chemical interactions that produce larger, precipitable complexes. These complexes darken the color of the tea infusion but can subsequently be removed through physical separation processes, thereby reducing the final tannin concentration in the extract (Table 2).

**Table 2** Water color absorbance (450 nm), polyphenol, and tannin concentrations in Uva black tea extracted in hard or soft water and filtered or centrifuged

|                           | Water color<br>450 nm    | Polyphenol<br>(GAE) mg/L | Tannin<br>mg/L       |
|---------------------------|--------------------------|--------------------------|----------------------|
| Soft water                | 0.599±0.010 <sup>c</sup> | 1002±17 <sup>c</sup>     | 811±17 <sup>c</sup>  |
| Soft water coffee filters | 0.584±0.010 <sup>c</sup> | 938±15 <sup>d</sup>      | 759±12 <sup>d</sup>  |
| Soft water centrifuge     | 0.568±0.016 <sup>d</sup> | 940±32 <sup>d</sup>      | 790±22 <sup>c</sup>  |
| Hard water                | 1.38±0.035 <sup>a</sup>  | 1059±19 <sup>a</sup>     | 901±18 <sup>a</sup>  |
| Hard water coffee filters | 1.36±0.018 <sup>a</sup>  | 1010±30 <sup>b</sup>     | 830±7.5 <sup>b</sup> |
| Hard water centrifuge     | 0.744±0.026 <sup>b</sup> | 901±35 <sup>b</sup>      | 784±23 <sup>b</sup>  |

**Remark:** GAE, gallic acid equivalents; ±, data are presented as mean ± SD;  $n = 9$ . The superscript letters a and b indicate comparisons within the hard water treatment groups, while c and d indicate comparisons within the soft water treatment groups ( $p < 0.05$ ).

**Table 3** Water color absorbance (450 nm) of black tea with different extraction solutions

| Condition                              | Absorbance (450 nm)         |
|----------------------------------------|-----------------------------|
| Soft water                             | 0.705 ± 0.072 <sup>c</sup>  |
| Hard water                             | 1.12 ± 0.14 <sup>a</sup>    |
| $\text{CaCl}_2$ (22.08 mg/100 mL) †    | 1.00 ± 0.066 <sup>ad</sup>  |
| $\text{CaCl}_2$ (11.73 mg/100 mL) ††   | 0.949 ± 0.12 <sup>ad</sup>  |
| $\text{MgSO}_4$ (12.87 mg/100 mL) †††  | 0.744 ± 0.14 <sup>bc</sup>  |
| $\text{CaCl}_2$ †+ $\text{MgSO}_4$ ††† | 1.12 ± 0.22 <sup>ad</sup>   |
| (22.08 mg/100 mL) + (12.87 mg/100 mL)  |                             |
| $\text{MgCl}_2$ (10.18 mg/100 mL) †††† | 0.641 ± 0.023 <sup>bc</sup> |

**Remark:** ±, Data are presented as mean ± SD;  $n = 9$ . †8.0 mg/100 mL as Ca; ††4.2 mg/100 mL as Ca; †††2.6 mg/100 mL as Mg. Superscript letters indicate significance compared to soft or hard water ( $p < 0.05$ ): a, no difference from hard water; b, significantly different from hard water; c, no difference from soft water; d, significantly different from soft water.

#### 4. Effect of metal ions on Uva black tea water coloration

Absorbance values were used as an indicator of the color intensity of Uva black tea extracted in different boiling solutions. Tea prepared with soft water exhibited an absorbance of 0.705, which was 37% lower than that of tea extracted with hard water (1.12;  $p > 0.05$ ). The absorbance of tea extracted with hard water was comparable to that obtained with calcium-containing solutions, being 12% higher than that observed for  $\text{CaCl}_2$  (22.08 mg/100 mL; absorbance 1.00) and 18% higher than that for  $\text{CaCl}_2$  (11.73 mg/100 mL; absorbance 0.949), although these differences were not statistically significant ( $p > 0.05$ ). Notably, the absorbance of tea extracted with hard water was identical to that observed for the combined  $\text{CaCl}_2 + \text{MgSO}_4$  solution (12.87 mg/100 mL; absorbance 1.12;  $p > 0.05$ ).

In contrast, the absorbance of tea prepared with soft water was similar to that observed in magnesium-containing solutions. Specifically, the absorbance of the soft-water extract was 5.2% lower than that of the  $\text{MgSO}_4$  solution (12.87 mg/100 mL; absorbance 0.744;  $p > 0.05$ ) and 10% higher than that of the  $\text{MgCl}_2$  solution (10.18 mg/100 mL; absorbance 0.641;  $p > 0.05$ ). All statistical comparisons were conducted using analysis of variance (ANOVA) followed by Tukey's post hoc test. Overall, these results indicate that the absorbance values of Uva black tea extracted with calcium-containing solutions were similar to those obtained with hard water, whereas extracts prepared with magnesium-containing solutions exhibited absorbance values comparable to those of soft water (Table 3). This corresponds to darker and lighter infusion colors for Uva black tea prepared with hard and soft water, respectively.

Black tea contains orange-red theaflavins and reddish-brown thearubigins, which are tannin-derived polyphenolic compounds responsible for its characteristic color. The CaO content increases with water hardness, and calcium ions can interact with tannins, particularly theaflavins and thearubigins, to form complexes that produce a darker, brownish coloration in black tea infusions (Tsuchiya, 2011). In contrast, magnesium ions have been reported to exhibit minimal interaction with tannins and do not significantly influence tea color unless present at concentrations exceeding approximately 97 ppm (Shimohashi & Terada, 1992). Because the magnesium ion concentration in Evian water is approximately 26 ppm, the color of black tea extracted in magnesium-containing solutions remained largely unchanged.

Therefore, calcium ions in hard water appear to play a significant role in influencing the color of Uva black tea extracts. Previous studies have also demonstrated that certain metal ions, such as iron, can form complexes with polyphenols (Zhang et al., 2023), and tannic acid has been reported to act as a sensor for several metal ions in aqueous systems, including  $\text{Cr}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  (Yıldız et al., 2021). In contrast, magnesium ions are rarely discussed in this context, suggesting that their complexation ability with polyphenolic compounds is comparatively weak and may be negligible under typical tea extraction conditions.

#### 5. Effect of pH on Uva black tea water coloration

The color intensity of Uva black tea was assessed by measuring the absorbance value. The absorbance of Uva black tea increased from 0.723 at pH 6 to 1.34 at pH 9, representing an 85.3% increase ( $p < 0.05$ , Fig. 5). At a Ca concentration of 0 mg/100 mL, the pH was 5.81 (Fig. 6), which decreased by 3.6% to 5.60 at a Ca concentration of 2.0 mg/100 mL ( $p < 0.05$ , t-test), and then increased by 16% to 6.52 at 8.0 mg/100 mL ( $p < 0.05$ , ANOVA and Tukey's post-hoc test). Thus, the pH of Ca solutions increased with the raise of Ca concentration. Hence, the color of black tea increased as the pH value became more alkaline. Under an acidic pH, the water color of black tea was bright orange, whereas under an alkaline pH, it became reddish brown compared to the deionized water leachate (Shimohashi & Terada, 1992). However, theaflavin in black tea becomes colorless under highly acidic conditions, e.g., when lemon is added to black tea. Therefore, the absorbance value of black tea water color increases with increased pH, as its color depends only on thearubigins in acidic solutions (Chemical Products PL Consultation Center, 2021). However, the pH of the Ca solution in the concentration range examined in this study was pH 5.60–6.52 (Fig. 6). As shown in Fig. 5, the absorbance of Uva black tea in this pH range was 0.723–0.803, which is slightly less than the absorbance of Uva black tea (1.00) extracted with  $\text{CaCl}_2$  (22.08 mg/100 mL: 8.0 mg/100 mL as Ca) solution (indicated in Table 4). Thus, pH does not greatly affect the color of water in hard water extraction. This study focused on Uva black tea, which contains high levels of tannins. However, the quality of tea leaves is influenced by various factors, such as origin and climate, making it impossible to use Uva black tea of consistent quality.

Additionally, hard and soft water contain trace amounts of metal ions other than Ca and Mg, which may also influence the results. This study examined the

removal of tannins; however, as tannins are a component of polyphenols, removing them will reduce the total amount of polyphenols. Therefore, it is necessary to remove an appropriate amount of tannins while leaving some polyphenols. Further studies remain warranted to address these limitations.

This study primarily focused on tannin extraction and removal, but the potential health effects of long-term high tannin intake should also be considered. Previous studies with humans found no significant changes in liver, kidney, or hematological parameters after consuming 480 mg for 6 months or 960 mg for 5 weeks (Maugeri et al., 2022). However, another clinical trial (29 Japanese adults) administered 1,000–2,500 mg/day of tannin-containing grape seed extract, resulting in a temporary and significant decrease in plasma iron concentration in two subjects, which normalized by the end of the trial (Maugeri et al., 2022). For comparison, the tannin content in common green and black teas has been reported as 101 mg TAE and 41.4 mg TAE per gram

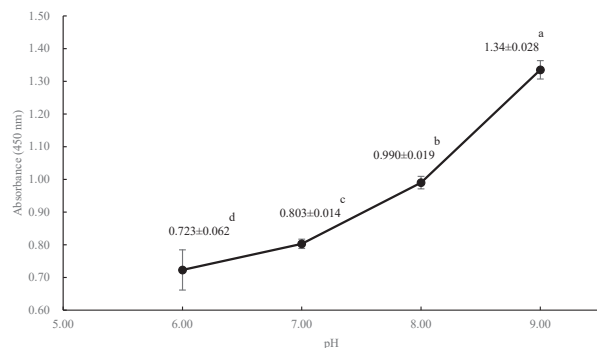
of dried leaves, respectively (Mohammad Taib et al., 2024). This suggests that tannin exposure from regular tea consumption is substantially lower. Nevertheless, these findings indicate that sustained intake of concentrated tannins may pose health risks.

## Conclusion

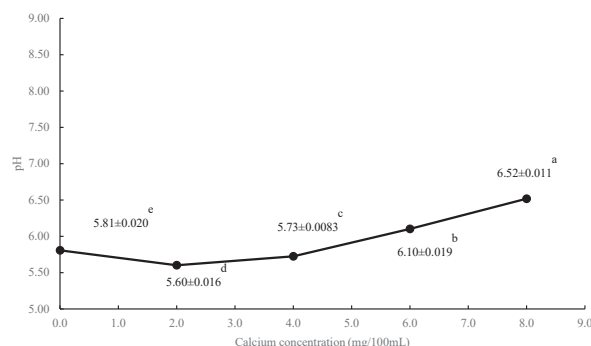
This study examined the effects of extraction conditions, water quality, treatment methods, and metal ions on tannin elution in Uva black tea. Tannin concentration increased with rising extraction temperature and time. Extraction with hard water resulted in a darker color (450 nm absorbance) compared to extraction with soft water, but no significant difference was observed in polyphenol and tannin concentrations. When filtered through a coffee filter or centrifuge, the filtrate color did not change after filter treatment with either hard or soft water, but the color became lighter in the supernatant after centrifugation. The polyphenol and tannin concentrations in the filtrate and supernatant tended to decrease. To investigate the effects of Ca and Mg ions, which are abundant in hard water, Ca and Mg were added alone or in combination, and the color was measured. The color in the Ca-added group was similar to that in hard water, whereas the color in the Mg-only-added group was similar to that in soft water. Because no increase in pH was observed even when the Ca ion concentration was varied with reference to the Ca ion concentration in hard water, it was shown that the increase in color was because of the presence of Ca but not a change in pH. Based on these results, combining Ca and tannin in hard water can be achieved using coffee filters or fine-mesh filters in typical households. In industrial settings where mechanical processing is feasible, centrifugation may prove a more efficient method for removing the flocculated complexes. Future research should focus on evaluating finer filtration media and enhancing removal efficiency by promoting the flocculation of metal ion-tannin complexes.

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**Fig. 5** Changes in absorbance of Uva black tea extract with increasing pH values. Letters with different superscripts mean significant difference ( $p < 0.05$ ). Error bars represent standard deviation;  $n = 5$ .



**Fig. 6** Relationship between Ca concentration and pH. Letters with different superscripts mean significant difference ( $p < 0.05$ ). Error bars represent standard deviation;  $n = 3$ .

## Conflicts of interest

There is no conflict of interest.

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used the services DeepL and Copilot for English translation and language refinement. The final content was reviewed and edited by the authors, who take full responsibility for the accuracy and integrity of the publication.

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