

Brewing conditions and low-temperature storage affect the total phenolics, caffeoylquinic acid content, and antioxidant activity of yerba mate infusion

Gholamreza Khaksar

Chulalongkorn University

Nantachaporn Chaichana

Chulalongkorn University

Kitipong Assatarakul

Chulalongkorn University

Supaart Sirikantaramas (✉ supaart.s@chula.ac.th)

Chulalongkorn University

Research Article

Keywords: caffeoylquinic acids (CQAs), dried leaf weight, light exposure, refrigerated storage, steeping time, yerba mate infusion

Posted Date: April 24th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2831137/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Herbal infusions exhibit diverse pharmacological effects, mainly attributed to the high content of phenolics (e.g., caffeoylquinic acids (CQAs)). Herein, we evaluated the content of CQAs in the methanolic extracts of model herbs, namely yerba mate (*Ilex paraguariensis*), stevia (*Stevia rebaudiana*), and *Pluchea indica*, showing that yerba mate had the highest total CQA content (108.05 ± 1.12 mg/g of dry weight). The analysis of yerba mate infusions prepared using different steeping times, dried leaf weights, and water temperature demonstrated that the amount of extracted CQAs was maximized (~ 175 mg per 150 mL) when 6 g of dried leaves were steeped in hot water for 10 min. Ten-day refrigerated storage induced no significant changes in the antioxidant activity and total phenolic and CQA contents of the infusion kept in a brown container but negatively affected the above parameters when kept in a clear container, suggesting the detrimental effect of light exposure. Our findings provide consumers, food scientists, and commercial producers with guidelines for optimizing the preparation and storage conditions of herbal infusions.

1. Introduction

As naturally occurring plant-derived substances, herbal medicines have long been used to treat numerous diseases and are mainly consumed in the form of herbal teas prepared as infusions (by steeping dried plant parts such as flowers, leaves, seeds, roots, and bark in hot water) or decoctions (by boiling herbs in water). Over the last decade, herbal teas have gained popularity among health-conscious consumers owing to their appealing flavors and health-beneficial properties such as antioxidant and therapeutic activities.

The health benefits provided by herbal teas are mainly attributed to their high contents of natural bioactive compounds such as phenolics. Caffeoylquinic acids (CQAs) are biologically important phenolics that are derived from hydroxycinnamic acid and exhibit numerous health-beneficial properties such as antioxidant, anti-inflammatory, anticancer, antidiabetic, antihypertensive, and antineurodegenerative activities (Santana-Gálvez et al., 2017). Based on the position, number, and identity of their acyl residues, these compounds, which are naturally produced by plants in response to biotic and abiotic stressors (Farah & Donangelo, 2006), can be categorized into three main groups, namely monocaffeoylquinic acids (monoCQAs: 3-CQA, 4-CQA, and 5-CQA; known as chlorogenic acids), dicaffeoylquinic acids (diCQAs: 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA), and feruloylquinic acids (FQAs: 3-FQA, 4-FQA, and 5-FQA) (Clifford and Wight, 1976; Kremr et al., 2015; Shin et al., 2015).

Previous studies have well documented the presence of CQAs in various herbal infusions. Marques and Farah (2009) identified and quantified CQAs in the infusions of 14 medicinal plants, with the highest CQA contents found in *Ilex paraguariensis* (yerba mate), *Bacharis genistelloides*, *Pimpinella anisum*, *Achyrocline satureioides*, *Camellia sinensis*, *Melissa officinalis*, and *Cymbopogon citratus* infusions. Based on the fact that the CQA content of toasted yerba mate was much lower than that of green yerba mate, the authors concluded that CQAs are heat-sensitive. Meinhart et al. (2018) profiled the content of

CQAs and caffeic acid in the infusions of 89 plants traditionally used and commercialized in Brazil, revealing that 93% of the tested infusions contained CQAs. The content of CQAs was highest for yerba mate (52.6 mg in 100 mL of infusion) and decreased in the order of yerba mate > *assa-peixe* (*Boehmeria caudata*) > white tea (*Camellia sinensis*) > winter's bark (*Drimys winteri*) > green tea (*Camellia sinensis*) > elderflower (*Sambucus nigra*). Chewchida and Vongsak (2019) reported the presence of mono- (~ 2.17 wt% 5-CQA) and diCQAs (~ 1.24 wt% 3,4-diCQA and ~ 1.93 wt% 3,5-diCQA) in the infusion of *Pluchea indica* (L.), a traditional Thai medicine widely used owing to its health-promoting properties. Karaköse et al. (2015) profiled the CQA content of stevia (*Stevia rebaudiana* Bertoni), a plant commonly used for sweetening, medicinal, pharmaceutical, and feeding purposes, showing that the corresponding leaf extract contained 5-CQA (~ 2.69 g/100 g of dry weight (DW)) and 4,5-diCQA (~ 1.68 g/100 g of DW).

Brewing conditions such as steeping temperature, steeping time, leaf particle size, and water/leaf mass ratio can significantly affect the antioxidant activity and total phenolic content of black and green teas (Khokhar and Magnúsdóttir, 2002; Komes et al., 2010; Venditti et al., 2010; Yuaan et al., 2015; Hajiaghaalipour et al., 2016; Chang et al., 2020). However, the effects of brewing parameters on the antioxidant activity and phenolic content of herbal infusions remain underexplored and have only been examined by Sentkowska et al. (2016), who investigated the effect of steeping time on the antioxidant activity and phenolic content of chamomile (*Matricaria chamomilla* L.) and St. John's wort (*Hypericum perforatum*) infusions. When the steeping time increased from 10 to 20 min, the CQA content of the chamomile infusion significantly increased, whereas that of the St. John's wort infusion remained almost unchanged. In contrast, the increase in steeping time did not affect the antioxidant activity of the chamomile infusion but increased that of the St. John's wort infusion. Considering the numerous health benefits of CQAs, a deeper understanding of the effects brewing conditions have on the CQA profiles of herbal infusions is of utmost importance for consumers and food scientists.

Another important factor possibly impacting the antioxidant activity and total phenolic content of herbal infusions is low-temperature storage (4°C). Previous studies have inconsistently reported the effects of low-temperature storage on the quality of teas and infusions. Jiménez-Zamora et al. (2016) reported the detrimental effect of three-month refrigerated storage on the antioxidant activity of 36 plant infusions traditionally consumed in Spain as infusions, revealing that this storage negatively affected antioxidant properties and induced a 50% loss of the total antioxidant activity. Labbé et al. (2008), Bazinet et al. (2010), and Ananingsih et al. (2013) reported no significant degradation of catechin or caffeine during the low-temperature storage of green tea. Moreover, Chang et al. (2020) reported no significant changes in the antioxidant activity and total phenolic content of black tea during 15-day storage at 4°C. However, to the best of our knowledge, the effect of refrigerated storage on the CQA content of herbal infusions has not been explored. When investigating the effect of storage on CQA content, one should consider temperature and light exposure, as CQAs can be degraded or isomerized at high temperatures or upon irradiation with light (Xue et al., 2016). During storage inside refrigerators, especially those with glass doors, herbal infusions can be periodically exposed to light.

Herein, we profiled the content of CQAs in the methanolic extracts of model herbs, namely yerba mate, stevia, and *P. indica*, and prepared a homemade infusion of the herb with the highest CQA content (yerba mate) to investigate the effect of brewing conditions (steeping time, steeping temperature, leaf/water mass ratio, and repetitive steeping) and low-temperature storage with and without light exposure on the content of CQAs therein. Thus, our work provides health-conscious consumers with a better understanding of the optimal conditions of herbal infusion preparation and storage while providing a first-time account of the potential effect of light exposure on the CQA content of a plant-based drink.

2. Materials And Methods

2.1. Reagents

Commercial standards (monoCQAs: 1-CQA, 3-CQA, 4-CQA, and 5-CQA; diCQAs: 1,3-diCQA, 1,4-diCQA, 1,5-diCQA, 3,4-diCQA, and 4,5-diCQA) were purchased from Biosynth Carbosynth®, UK (purity $\geq 98.0\%$), and 3,5-diCQA was purchased from TransMIT GmbH PlantMetaChem (Germany). Puerarin (internal standard) was obtained from Sigma-Aldrich, Inc., St. Louis, MO, USA (purity $\geq 98.0\%$). Acetonitrile and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). Ultrapure water prepared using the Milli-Q system (Millipore, Billerica, MA, USA) was used in all experiments. All reagents used to determine antioxidant activity and total phenolic content were obtained from Sigma-Aldrich, Inc., St. Louis, MO, USA.

2.2. Plant materials

Plant samples were obtained from reliable commercial sources in Bangkok, Thailand in the form of dried leaves, which is how they are usually consumed. Three brands of each plant were examined. All samples were analyzed without further drying to maintain the original content of CQAs.

2.3. Preparation of methanolic extracts

Samples were ground to a fine powder passing through a 0.75-mm sieve using a mixer mill (MM 400, Retsch GmbH) at 30 Hz for 1 min. A 20-mg aliquot (DW) of each ground sample was extracted with 1 mL of 80 vol% aqueous methanol containing puerarin (0.05 g L^{-1}) as an internal standard upon vigorous 15-min shaking at 1500 rpm and 15°C (Eppendorf Thermomixer® C, Eppendorf, USA). The resulting mixtures were centrifuged at 12000 *g* and 4°C for 15 min using a table-top centrifuge (5415 R, Eppendorf, USA), and the supernatants were collected, filtered through a 0.2- μm nylon syringe filter, and injected into a high-performance liquid chromatograph for CQA analysis (Khaksar et al., 2021).

2.4. Infusion preparation

The effects of brewing conditions on the antioxidant activity and CQA content were investigated for the infusion of the plant with the highest CQA content (yerba mate).

2.4.1. Effects of water/leaf mass ratio and steeping time

Dried yerba mate leaves (2 (equivalent to plant mass in a commercial sachet), 4, 6, and 8 g) were added to 150 mL (volume of a medium-size cup) of hot water (~ 90°C) and steeped for 5 min (according to most manufacturers). The resulting infusions were filtered through paper (Whatman No. 1), immediately cooled in an ice bath, filtered through a 0.2-µm nylon syringe filter, and analyzed (chromatography, total phenolic content, and antioxidant activity). To further investigate the effect of steeping time (10, 15, and 30 min) on CQA content, the corresponding infusions were filtered and analyzed as described in the related sections. The thus identified optimal brewing conditions were used for subsequent experiments.

2.4.2. Effects of repeated infusion preparation

Many consumers repeat the infusion preparation by adding water to steeped leaves. Accordingly, to examine the effect of this process on CQA content, a yerba mate infusion was prepared using hot water under the optimal conditions obtained in section 2.4.1. After filtering, the infusion preparation was repeated by adding 150 mL of hot water to the steeped leaves (second cycle), and this process was repeated one more time (third cycle). After each cycle, the infusion was filtered through a 0.2-µm nylon syringe filter and analyzed (chromatography, total phenolic content, and antioxidant activity) as described in the related sections.

2.4.3. Effects of steeping temperature

Steeping with cool water is a common practice among yerba mate consumers, especially in Paraguay and Southern Brazil (<https://mateina.ca/pages/preparation>). Accordingly, to reproduce these conditions and investigate the effect of steeping temperature on the CQA content, total phenolic content, and antioxidant activity, we prepared an infusion using cool water (~ 27°C) under the optimal conditions obtained in section 2.4.1, subjected it to the corresponding analyses, and compared the results with those obtained for the infusion prepared using hot water.

2.5. Effects of low-temperature storage

To investigate the effect of storage under simulated refrigeration conditions on the antioxidant activity, total phenolic content, and total CQA content, we prepared a yerba mate infusion under the optimal conditions obtained in section 2.4.1. The infusion was filtered, processed as described above, and stored inside brown (dark condition) and transparent (normal condition) air-tight glass containers inside a glass-door refrigerator (4°C) for 10 days. After 1, 3, 5, 7, and 10 days of storage, the samples were filtered through a 0.2-µm nylon syringe filter and analyzed.

2.6. Antioxidant activity determination

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was assessed following the method of Brand-Williams et al. (1995) with some modifications (Khaksar et al., 2019). The DPPH working solution was prepared by mixing 10 mL of the stock solution (24 mg of DPPH dissolved in 100 mL of methanol) with 45 mL of methanol. A 50-µL aliquot of the infusion was mixed with the DPPH working solution (950 µL) and incubated for 1 h in the dark. Absorbance at 515 nm (A_{515}) was read using a microplate reader, and DPPH radical scavenging activity was calculated as

DPPH radical scavenging activity (%) = $100 \times (A - A_1 + A_2)/A$,

where A (absorbance) is the A_{515} of the DPPH solution without the infusion, A_1 is the A_{515} of the DPPH solution with the infusion, and A_2 is the A_{515} of the infusion.

2.7. Total phenolic content

Total phenolic content was measured according to the Folin-Ciocalteu method (Swain and Hillis, 1959). A 150- μ L aliquot of the infusion, 2400 μ L of ultrapure water, and 150 μ L of 0.5 N Folin–Ciocalteu reagent were vigorously mixed and left to react for 30 min at room temperature (RT) ($\sim 28^\circ\text{C}$). The mixture was then supplemented with 300 μ L of 1 N Na_2CO_3 solution, vortexed, and left to stand at RT for 2 h in the dark. Absorbance at 725 nm (A_{725}) was then read using a microplate reader and converted into total phenolic acid content expressed as g gallic acid equivalent (GAE) per 150 mL.

2.8. Total microbial count determination and color analysis

For total microbial count determination, each infusion sample was serially diluted (10^{-2} – 10^{-8}) with autoclaved water containing 0.1% peptone. A 1-mL aliquot of each diluted sample was placed on a Luria-Bertani agar plate and incubated at 37°C for two days to obtain the total aerobic bacterial count. For total yeast and mold count determination, a 1-mL aliquot of each diluted sample was placed on a potato dextrose agar plate and incubated at 30°C for three to five days. The results were expressed as log colony-forming units (CFU) per mL of sample.

For color analysis, three color parameters, namely L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness), were measured using a colorimeter (CR-410 chroma meter, Minolta, Japan). Color difference (ΔE) was calculated using the freshly prepared infusion as a control:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$

2.9. Ultrapformance liquid chromatography (UPLC)

The CQA profiling of infusions was performed using an UltiMate 3000 UPLC system coupled with a Dionex UltiMate DAD 3000 detector (Thermo Fisher Scientific, Waltham, MA, USA) and equipped with a Kinetex EVO C18 column (250 mm $\times$ 4.6 mm, 5 μ m) (Phenomenex, USA) at a detection wavelength of 325 nm following the method described in our previous study (Khaksar et al., 2021). The mobile phase was composed of water with 0.1 vol% trifluoroacetic acid (TFA) (pH 2.4; eluent A) and acetonitrile with 0.1 vol% TFA (eluent B). The following gradient was used: 0–5% B (0.1–5 min), 5–15% B (5–40 min), 15–100% B (40–50 min), 100–5% B (50–60 min). The flow rate and injection volume equaled 1 mL/min and 10 μ L, respectively. Peaks matching the retention times and UV spectra of commercial standards were identified as CQAs. Puerarin was used as an internal standard. Each CQA was quantified according to its calibration curve in the range of 1.30–500 μ g/mL. More information on the employed CQA standards, including standard curves and equations, limit of detection, and limit of quantification, is presented in Supplementary Data (Supplementary Fig. S1 and Supplementary Table S1).

2.10. Statistical analysis

The acquired data were subjected to statistical analysis using SPSS software v. 20 (SPSS Inc., IBM) and presented as means $\pm$ standard deviations of three independent replicates. Statistical comparisons of means were carried out using one-way ANOVA followed by Duncan's multiple range test or Student's *t*-test at the 0.05 confidence level.

3. Results

3.1. CQA profiles of methanolic extracts

Six CQAs (3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) were identified and quantified in the investigated plants (Table 1). The total CQA content decreased in the order of yerba mate (108.05 ± 1.12 mg/g DW basis) > stevia (79.26 ± 0.74 mg/g DW basis) > *P. indica* (64.59 ± 1.18 mg/g DW basis). Notably, whereas yerba mate and stevia contained all six of the above CQAs, *P. indica* contained only five (i.e., did not contain 4-CQA). The contributions of mono- and diCQAs to the total CQA content depended on the plant, i.e., the major contributor was identified as monoCQAs for yerba mate and stevia and as diCQAs for *P. indica*. The distribution of monoCQAs also depended on the plant, i.e., 3-CQA was the most abundant monoCQA in yerba mate, while 5-CQA was most abundant in stevia and *P. indica*. Regarding diCQAs, 3,5-diCQA was the major isomer in all samples. Figure 1 presents the chromatograms of the analytical standards and the yerba mate sample.

Table 1
CQA contents of methanolic plant extracts.

Sample	3-CQA	4-CQA	5-CQA	3,5-diCQA	3,4-diCQA	4,5-diCQA	Total CQA
<i>Ilex paraguariensis</i> (brand A)	25.11 ± 0.97 ^a	18.11 ± 0.44 ^a	23.11 ± 0.44 ^a	21.22 ± 1.01 ^a	7.11 ± 0.55 ^b	12.22 ± 0.41 ^a	108.05 ± 1.12 ^a
<i>Ilex paraguariensis</i> (brand B)	21.69 ± 1.02 ^b	14.90 ± 1.01 ^b	18.88 ± 0.67 ^b	21.01 ± 0.37 ^a	7.53 ± 0.41 ^b	12.22 ± 0.85 ^a	98.21 ± 0.86 ^b
<i>Ilex paraguariensis</i> (brand C)	21.55 ± 0.97 ^b	15.02 ± 0.44 ^b	18.01 ± 1.11 ^b	20.35 ± 0.73 ^a	7.33 ± 0.55 ^b	12.01 ± 0.86 ^a	97.69 ± 0.77 ^b
<i>Stevia rebaudiana</i> (brand A)	16.66 ± 0.65 ^c	9.11 ± 0.60 ^c	19.61 ± 0.31 ^b	16.16 ± 0.54 ^b	6.43 ± 0.51 ^b	9.22 ± 0.24 ^b	78.79 ± 1.07 ^c
<i>Stevia rebaudiana</i> (brand B)	17.11 ± 0.43 ^c	9.04 ± 0.51 ^c	19.22 ± 0.31 ^b	16.79 ± 0.36 ^b	6.21 ± 0.61 ^b	9.55 ± 0.30 ^b	79.11 ± 0.75 ^c
<i>Stevia rebaudiana</i> (brand C)	17.94 ± 0.12 ^c	9.12 ± 0.66 ^c	19.11 ± 0.23 ^b	17.01 ± 0.91 ^b	6.11 ± 0.81 ^b	8.97 ± 0.34 ^b	79.26 ± 0.74 ^c
<i>Pluchea indica</i> (brand A)	3.11 ± 0.82 ^d	nd	23.97 ± 0.24 ^a	17.22 ± 0.63 ^b	10.65 ± 0.51 ^a	6.07 ± 0.14 ^c	63.11 ± 1.32 ^d
<i>Pluchea indica</i> (brand B)	3.71 ± 0.21 ^d	nd	23.16 ± 0.31 ^a	17.11 ± 0.69 ^b	11.01 ± 0.93 ^a	5.99 ± 0.21 ^c	62.66 ± 0.96 ^d
<i>Pluchea indica</i> (brand C)	4.66 ± 0.80 ^d	nd	24.01 ± 0.83 ^a	17.65 ± 0.55 ^b	11.11 ± 0.95 ^a	5.89 ± 0.11 ^c	64.59 ± 1.18 ^d
Data are presented as the means ± standard deviations of three independent replicates and expressed in mg/g of DW. For each compound, values in the same column followed by different superscript letters are significantly different according to Duncan's multiple-range test ($p < 0.05$). nd: not detected (detection limit = 1.09 µg/mL).							

For each plant, the observed CQA distributions were similar across different commercial brands. No significant variation in the contents of individual isomers was found for stevia and *P. indica*, whereas for yerba mate, monoCQA isomers were significantly more abundant in brand A than in brands B and C (Table 1), which was ascribed to the effects of cultivation and climatic conditions and/or processing. In addition, we did not observe significant differences in total CQA content between methanolic and aqueous extracts of yerba mate.

3.2. Effects of brewing conditions

3.2.1. Effects of water/leaf mass ratio and steeping time

For a 5-min steeping time (recommended by most manufacturers), the antioxidant activity (Fig. 2A), total phenolic content (Fig. 2B), and total CQA content (Fig. 2C) of yerba mate infusions increased with increasing dried leaf weight, peaking at a dried leaf weight of 8 g. However, for steeping times of 10, 15, and 30 min, these parameters were maximized at a dried leaf weight of 6 g and did not change when this weight increased to 8 g (Fig. 2). At a constant dried leaf weight, an increase in steeping time from 5 to 10 min significantly increased antioxidant activity, total phenolic content, and total CQA content, whereas a further steeping time extension to 15 and 30 min had no effect (Fig. 2). Thus, the optimum brewing conditions corresponded to a steeping time of 10 min and a dried leaf weight of 6 g.

3.2.2. Effects of repeated infusion preparation

Repeated infusion preparation negatively affected antioxidant activity (Fig. 3A), total phenolic content (Fig. 3B), and CQA content (Fig. 3C), all of which were minimized after the third cycle. Thus, the infusion with the highest antioxidant ability, total phenolic content, and total CQA content was prepared using a single steeping cycle.

3.2.3. Effects of steeping temperature

Given that CQAs are heat sensitive, we investigated the effects of steeping temperature on the quality of the resulting infusion. Surprisingly, the antioxidant activity, total phenolic content, and total CQA content of the infusion prepared using cool water were significantly lower than those of the infusion prepared using hot water (Fig. 4).

3.3. Effects of simulated refrigerated storage

3.3.1. Effects on antioxidant activity, total phenolic content, and total CQA content

The refrigerated storage and on-demand consumption of home-made infusions is a regular practice among consumers. Herein, the storage of the yerba mate infusion in a brown glass container (dark condition) at 4°C did not affect its quality, i.e., during 10-day storage, the antioxidant activity (Fig. 5A), total phenolic content (Fig. 5B), and total CQA content (Fig. 5C) did not significantly deviate from those of the control sample (freshly prepared infusion). However, low-temperature storage negatively affected infusion quality when a transparent glass container (normal conditions) was used. As shown in Figs. 5A, 5B, and 5C, all measured parameters remained unchanged until day seven, significantly decreasing on day 10.

3.3.2. Effects on total microbial count and color

Ten-day storage at 4°C did not affect the total microbial count, i.e., during the storage period, the counts of total aerobic bacteria (Supplementary Fig. S2A) and yeast/mold (Supplementary Fig. S2B) did not

significantly deviate from those of the control sample. Similarly, no significant changes were observed for L^* , a^* , and b^* (Supplementary Table S2). The color difference (ΔE) did not significantly vary during storage and remained within the range of 0–1, suggesting an invisible difference.

4. Discussion

Yerba mate, widely consumed in South America, is well known for its high content of phenolics such as CQAs (Clifford and Ramirez-Martinez, 1990; Bastos et al., 2005). Herein, six CQA isomers (three monoCQAs and three diCQAs) were identified and quantified in this plant. MonoCQAs accounted for 60% (on average) of the total CQA content and were mainly represented by 3-CQA, while diCQAs were mainly represented by 3,5-diCQA. Thus, our findings are in line with those of Clifford and Ramirez-Martinez (1990), Marques and Farah (2009), and Meinhart et al. (2018), who also reported the presence of six CQA isomers in yerba mate and showed that 3-CQA and 3,5-diCQA were the most abundant mono- and diCQAs, respectively. Notably, Marques and Farah (2009) reported the presence of caffeine in the methanolic extract of yerba mate (~ 15 mg per 100 g of DW), which is something caffeine-sensitive consumers should be aware of. The methanolic and aqueous extracts of yerba mate had similar total CQA contents, in line with the report of Marques and Farah (2009), which suggested that a satisfactory CQA extraction efficiency is achieved during the home preparation of yerba mate infusions. Data on the CQA content of beverages (except for coffee, which is well known to be rich in CQAs) are scarce. Jeon et al. (2019) determined the total content of CQAs in instant coffee (100%), ready-to-drink coffee (non-milk added coffee), and ground coffee (unblended and roasted) as ~ 80.7, 75.02, and 217 mg per 150 mL. Greenrod et al. (2005) determined the total phenolic content of red wine (which is a good source of phenolics) as ~ 100 mg per 150 mL. The CQA-rich yerba mate commonly consumed as a homemade infusion deserves attention in view of the pharmacological activity of CQAs. However, one cannot claim that all plants with high CQA contents are good sources of CQAs for humans, as the influence of the food matrix on CQA bioavailability is still unknown. Moreover, it would not be correct to assert that the lower CQA contents of some herbal infusions make them not important to humans, as the metabolism and requirements of these compounds seem to vary amongst individuals, with no dietary recommendations currently established.

During repeated steeping, CQA content was minimized at the end of the third cycle (~ 75 mg per 150 mL) (Fig. 3) but was still comparable to that of instant and ready-to-drink coffee, which suggested that satisfactory CQA extraction was achieved even upon repeated infusion preparation. Altogether, ~ 375 mg of CQAs (~ 58% of the total content in 6 g) was extracted over three cycles.

Herbal infusion preparation using cool water is a common practice among consumers, e.g., infusions prepared by 2-h steeping at ~ 25°C have become popular in Taiwan (Venditti et al., 2010). Herein, the antioxidant activity, total phenolic content, and total CQA content of the cold-brewed infusion were significantly lower than those of the hot-brewed infusion (Fig. 4), in agreement with the results previously obtained for the total phenolic content and antioxidant activity of white, green, and black tea infusions prepared with cold water (Khokhar and Magnusdottir, 2002; Venditti et al., 2010; Yuann et al., 2015;

Hajiaghaalipour et al., 2016). The effect of steeping temperature on CQA content observed herein was attributed to the increased solubility of CQAs in warm water (De Maria et al., 1998). Moreover, Dibert et al. (1989) reported that the diffusivity of chlorogenic acids in water increases with increasing temperature. A rise in plant cell permeability with increasing temperature may also increase the transference rate of CQAs. Notably, the preparation of the yerba mate infusion under optimum brewing conditions (steeping of 6 g of dried leaves for 10 min) using hot water did not have any detrimental effect on CQA content.

Although the refrigerated storage and on-demand consumption of homemade infusions is a regular practice, health-conscious consumers are often concerned about the effects of storage conditions on the nutritional qualities of infusions. Therefore, we investigated the effect of refrigerated storage on the antioxidant activity, total phenolic content, and CQA content of a homemade yerba mate infusion over a 10-day period, revealing that these parameters were not affected in the case when storage was performed in a brown glass container but experienced deterioration when storage was performed in a transparent glass container (Fig. 5). Thus, our findings strongly suggest that exposure to light has a detrimental effect on the antioxidant activity, total phenolic content, and total CQA content of infusions. To the best of our knowledge, this is the first report on the effect of light on the CQA content of a drink.

Color stability is an important quality characteristic of drinks during storage. Unlike in the case of CQA content, exposure to light did not affect the color stability and total microbial count of the infusion during storage.

Herein, we utilized targeted metabolomics to profile the CQA contents of yerba mate infusions prepared under different conditions, as CQAs exhibit numerous health-beneficial properties and are the predominant phenolic compounds in yerba mate. This point justifies the application of targeted metabolite profiling in the present study. However, other bioactive compounds possibly contributing to the nutritional quality and health benefits of yerba mate also deserve attention, and future work should therefore focus on the use of non-targeted metabolomics to profile the bioactive compounds of yerba mate infusions prepared under different conditions.

In summary, we profiled the content of CQAs in the methanolic extracts of three medicinal plants, namely yerba mate, stevia, and *P. indica*, showing that yerba mate had the highest total CQA content. Subsequently, we prepared a homemade infusion of yerba mate and investigated the effects of brewing conditions (steeping time and temperature, leaf/water mass ratio, and repetitive steeping) and refrigerated storage (4°C, light and dark conditions) on its antioxidant activity, total phenolic content, and total CQA content. Except for low-temperature storage under dark conditions, which did not have any detrimental effect, all factors significantly affected antioxidant activity, total phenolic content, and CQA content. Considering the numerous health benefits of CQAs, our findings provide health-conscious consumers and food scientists with an improved understanding of the optimal conditions for the preparation and storage of herbal infusions.

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

S.S. and K.A. conceived the study. G.K. and N.C. performed the experiments. K.A. contributed analysis tools or data. S.S. and G.K. analyzed and interpreted the data and drafted the manuscript. All authors edited, read, and approved the final manuscript.

Funding

This research was supported by the Center of Excellence in Molecular Crop (to S.S.) and the Ratchadapisek Somphot Fund for Postdoctoral Fellowship (to G.K.), Chulalongkorn University. The funding bodies were not involved in the design of the study, sample collection, data analyses, data interpretation, and the manuscript writing.

Acknowledgments

We thank Ms. Rawisada Pholsin and Ms. Pornpassorn Chulalaksananukul for their assistance with color and total microbial count analyses.

Ethics Approval

Not applicable

Consent to Participate

Not applicable

Consent for Publication

Not applicable

Data Availability

All data generated or analyzed for this study are included in this published article and its supplementary information files.

References

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

2. Bastos, D. H. M., Fornari, A. C., de Queiroz, Y. S., Soares, R. A. M., & Torres, E. A. F. S. (2005). The Chlorogenic Acid and Caffeine Content of Yerba Maté (*Ilex paraguariensis*) Beverages. *Acta Farm. Bonaerense*, 24, 91-5.
3. Bazinet, L., Araya-Farias, M., Doyen, A., Trudel, D., & Têtu, B. (2010). Effect of process unit operations and long-term storage on catechin contents in EGCG-enriched tea drink. *Food Research International*, 43, 1692-1701.
4. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*, 28, 25–30.
5. Chang, M-Y., Lin, Y-Y., Chang, Y-C., Huang, W-Y., Lin, W-S., Chen, C-Y., Huang, S-L., & Lin, Y-S. (2020). Effects of Infusion and Storage on Antioxidant Activity and Total Phenolic Content of Black Tea. *Applied Sciences* 10, 2685.
6. Chewchida, S., & Vongsak, B. (2019). Simultaneous HPTLC quantification of three caffeoylquinic acids in *Pluchea indica* leaves and their commercial products in Thailand. *Revista Brasileira de Farmacognosia*, 29, 177-181.
7. Clifford, M. N., & Ramirez-Martinez, J. R. (1990). Chlorogenic acids and purine alkaloids contents of Mate (*Ilex paraguariensis*) leaf and beverage. *Food Chemistry*, 35, 13–21.
8. Clifford, M. N., & Wight, J. (1976). The measurement of feruloylquinic acids and caffeoylquinic acids in coffee beans. Development of the technique and its preliminary application to green coffee beans. *Journal of the Science of Food and Agriculture*, 27, 73-84.
9. De Maria, C. A. B., Trugo, L. C., De Mariz, E., Miranda, L. S., & Salvador, E. (1998). Stability of 5-caffeoylquinic acid under different conditions of heating. *Food Research International*, 31, 475-477.
10. Dibert, K., Cros, E., & Andrieu, J. (1989). Solvent extraction of oil chlorogenic acid from green coffee. Part II: kinetic data. *Journal of Food Engineering*, 10, 199-214.
11. Farah, A., & Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology*, 18, 23-36.
12. Greenrod, W., Stockley, C. S., Burcham, P., Abbey, M., & Fenech, M. (2005). Moderate acute intake of de-alcoholised red wine, but not alcohol, is protective against radiation-induced DNA damage ex vivo – Results of a comparative in vivo intervention study in younger men. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 591, 290-301.
13. Hajiaghaalipour, F., Sanusi, J., & Kanthimathi, M. S. (2016). Temperature and time of steeping affect the antioxidant properties of white, green, and black tea infusions. *Journal of Food Science*, 81, 246-254.
14. Jeon, J. S., Kim, H. T., Jeong, I. H., Hong, S. R., Oh, M. S., Yoon, M. H., Shim, J. H., Jeong, J. H., & Abd El-Aty, A. M. (2019). Contents of chlorogenic acids and caffeine in various coffee-related products. *Journal of Advanced Research*, 17, 85-94.
15. Jiménez-Zamora, A., Delgado-Andrade, C., & Rufián-Henares, J. A. (2016). Antioxidant capacity, total phenols and color profile during the storage of selected plants used for infusion. *Food Chemistry*, 199, 339-46.

16. Karaköse, H., Jaiswal, R., Deshpande, S., & Kuhnert, N. (2015). Investigation of the photochemical changes of chlorogenic acids induced by ultraviolet light in model systems and in agricultural practice with *Stevia rebaudiana* cultivation as an example. *Journal of Agricultural and Food Chemistry*, *63*, 3338-47.
17. Khaksar, G., Assatarakul, K., & Sirikantaramas, S. (2019). Effect of cold-pressed and normal centrifugal juicing on quality attributes of fresh juices: do cold-pressed juices harbor a superior nutritional quality and antioxidant capacity? *Heliyon*, *5*, e01917.
18. Khaksar, G., Cheevarungrapakul, K., Boonjing, P., & Sirikantaramas, S. (2021). Sprout caffeoylquinic acid profiles as affected by variety, cooking, and storage. *Frontiers in Nutrition*, *8*, 748001.
19. Khokhar, S., & Magnusdottir, S. G. M. (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *Journal of Agricultural and Food Chemistry*, *50*, 565-570.
20. Komes, D., Horžić, D., Belščak, A., Ganić, K. K., & Vulić, I. (2010). Green tea preparation and its influence on the content of bioactive compounds. *Food Research International*, *43*, 167-176.
21. Kremr, D., Cocovi-Solberg, D.J., Bajeroová, P., Ventura, K., & Miró, M. (2015). On-line monitoring of in-vitro oral bioaccessibility tests as front-end to liquid chromatography for determination of chlorogenic acid isomers in dietary supplements. *Talanta*.
22. Labbé, D., Têtu, B., Trudel, D., & Bazinet, L. (2008). Catechin stability of EGC- and EGCG-enriched tea drinks produced by a two-step extraction procedure. *Food Chemistry*, *111*, 139-143.
23. Marques, V., & Farah, A. (2009). Chlorogenic acids and related compounds in medicinal plants and infusions. *Food Chemistry*, *113*, 1370-1376,
24. Meinhart, A. D., Caldeirao, L., Damin, F. M., Filho, J. T., & Godoy H. T. (2018). Analysis of chlorogenic acids isomers and caffeic acid in 89 herbal infusions (tea). *Journal of Food Composition and Analysis*, *73*, 76-82.
25. Santana-Gálvez, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2017). Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. *Molecules*, *22*, 358.
26. Sentkowska, A., Biesaga, M., & Pyrzynska, K. (2016). Effects of brewing process on phenolic compounds and antioxidant activity of herbs. *Food Science and Biotechnology*, *25*, 965-970.
27. Shin, H.S., Satsu, H., Bae, M.J., Zhao, Z., Ogiwara, H., Totsuka, M., & Shimizu, M. (2015). Anti-inflammatory effect of chlorogenic acid on the IL-8 production in Caco-2 cells and the dextran sulphate sodium-induced colitis symptoms in C57BL/6 mice. *Food Chemistry*, *168*, 167-175.
28. Venditti, E., Bacchetti, T., Tiano, L., Carloni, P., Greci, L., & Damiani, E. (2010). Hot vs. cold water steeping of different teas: Do they affect antioxidant activity? *Food Chemistry*, *119*, 1597-1604.
29. Xue, M., Shi, H., Zhang, J., Liu, Q. Q., Guan, J., Zhang, J. Y., & Ma, Q. (2016). Stability and degradation of caffeoylquinic acids under different storage conditions studied by high-performance liquid chromatography with photo diode array detection and high-performance liquid chromatography with

electrospray ionization collision-induced dissociation tandem mass spectrometry. *Molecules*, 21, 948.

30. Yuann, J. M. P., Wu, J. Y., Chang, H. H., & Liang, J. Y. (2015). Effects of temperature and water steeping duration on antioxidant activity and caffeine content of tea. *MC-Transaction on Biotechnology*, 7, 22–32.

Figures

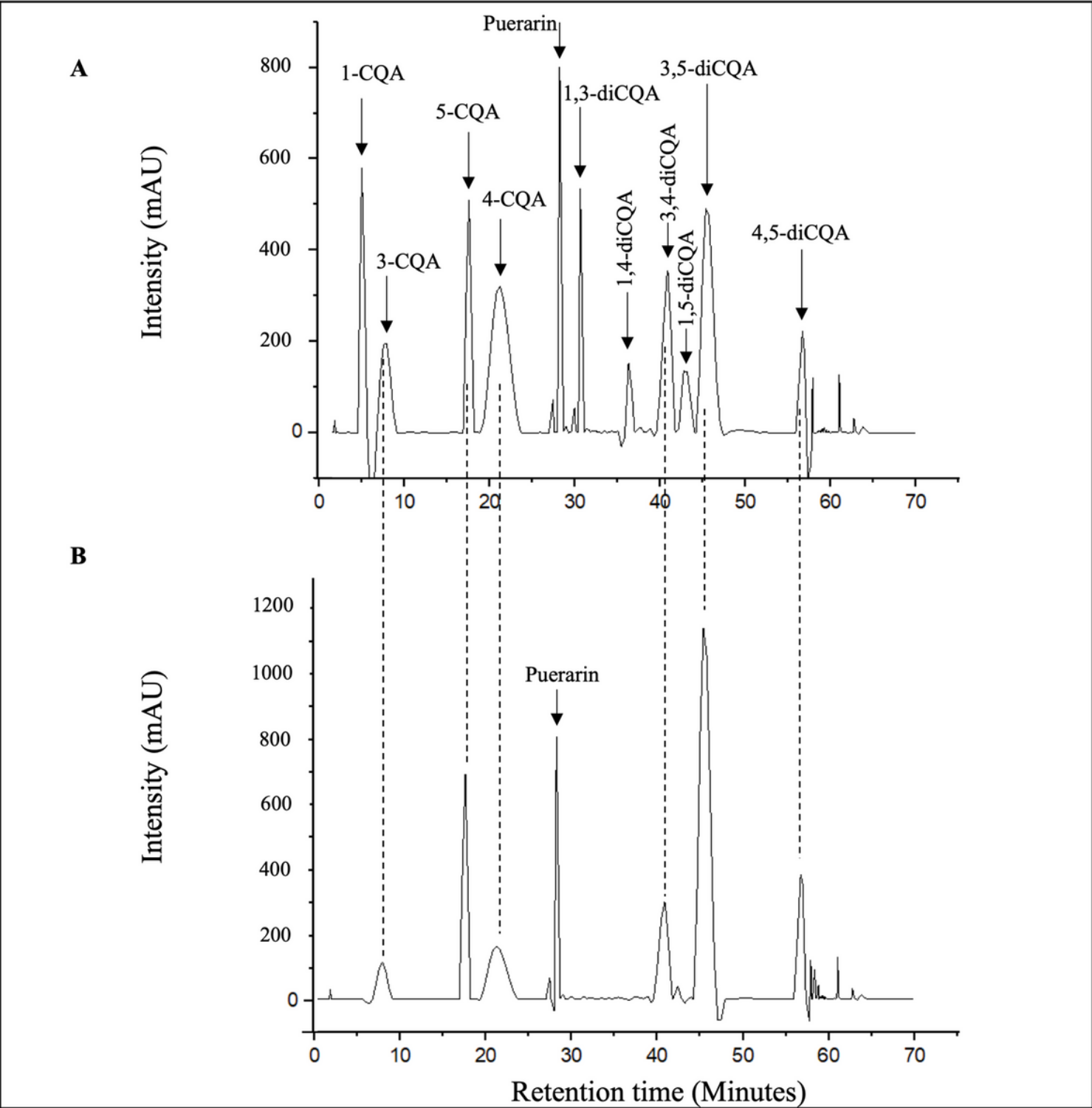


Figure 1

Chromatograms of (A) the analytical standards and (B) the yerba mate sample.

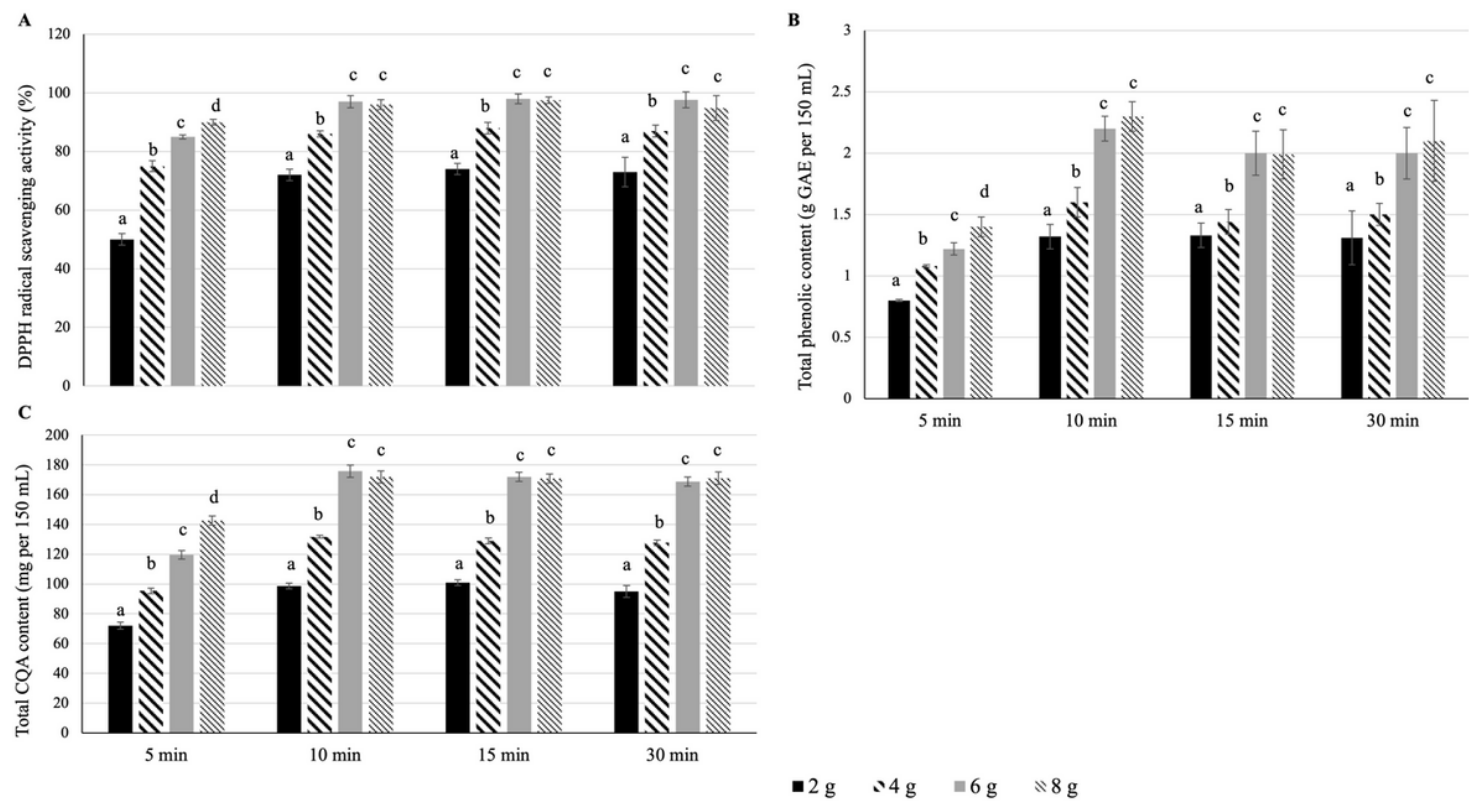


Figure 2

Effects of the water/leaf mass ratio and steeping time on the (A) antioxidant activity, (B) total phenolic content, and (C) total CQA content of yerba mate infusions. Error bars represent means ± standard deviations ($n = 3$). For each steeping time, comparisons are made among different leaf weights, bars with different letters differ significantly according to Duncan's multiple range test ($p < 0.05$).

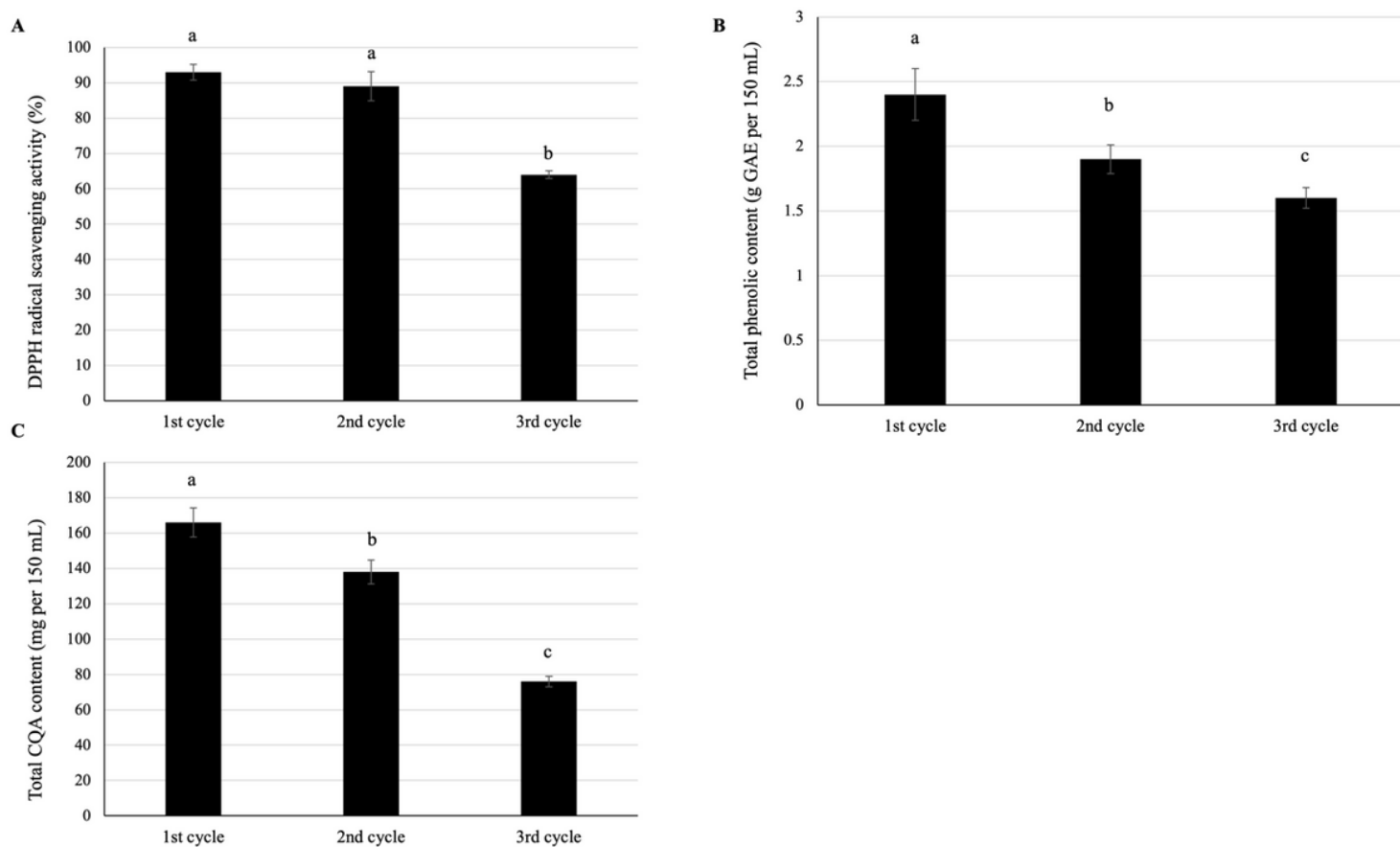


Figure 3

Effect of repeated preparation on the (A) antioxidant activity, (B) total phenolic content, and (C) total CQA content of yerba mate infusions. Error bars represent means $\pm$ standard deviations ($n = 3$). For each parameter, comparisons are made among three cycles, bars with different letters differ significantly according to Duncan's multiple range test ($p < 0.05$).

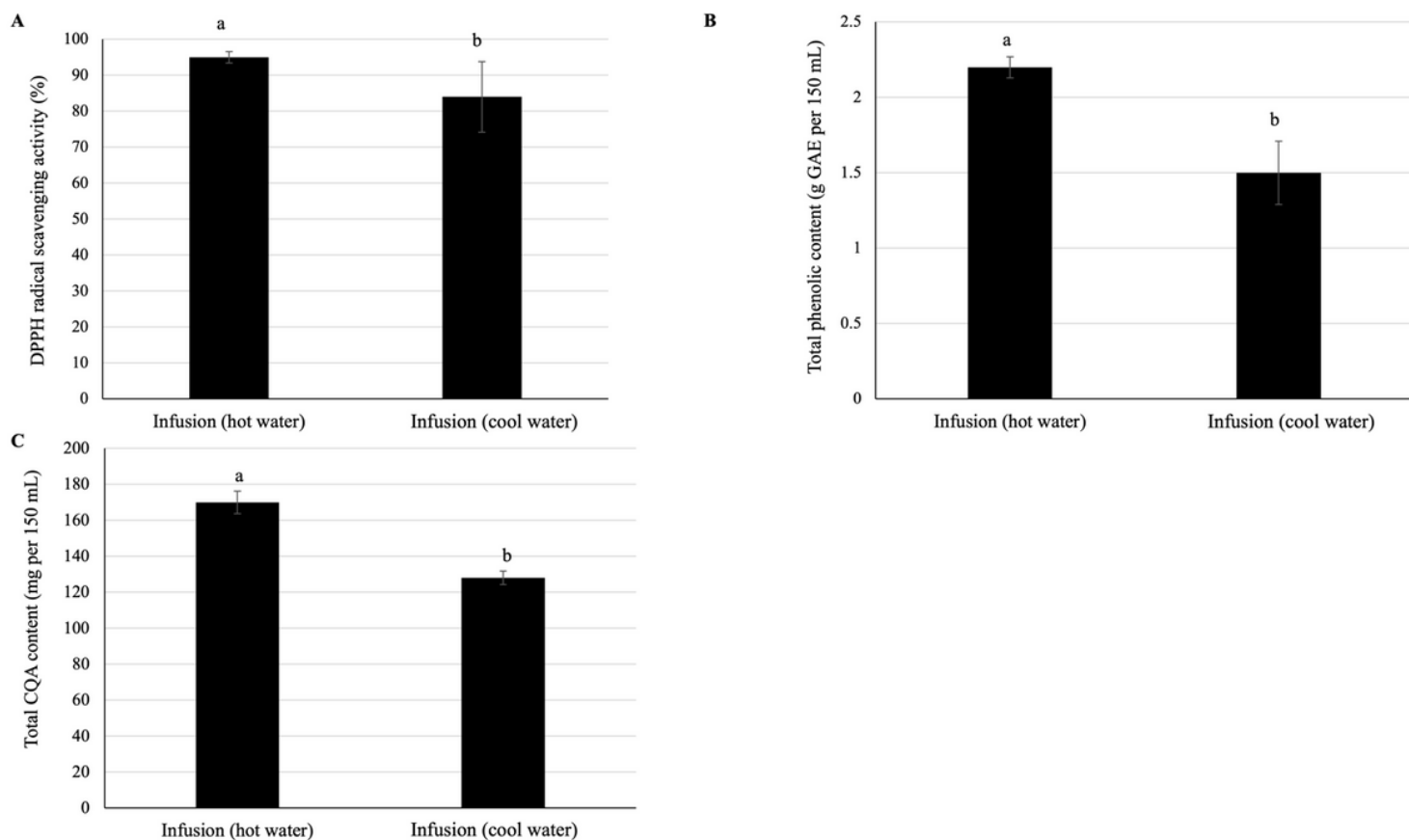


Figure 4

Effect of steeping temperature (90 and 27 °C) on the (A) antioxidant activity, (B) total phenolic content, and (C) total CQA content of yerba mate infusions. Error bars represent means $\pm$ standard deviations ($n = 3$). For each parameter, comparisons are made between infusions prepared using hot and cold water, bars with different letters differ significantly according to Student's t -test ($p < 0.05$).

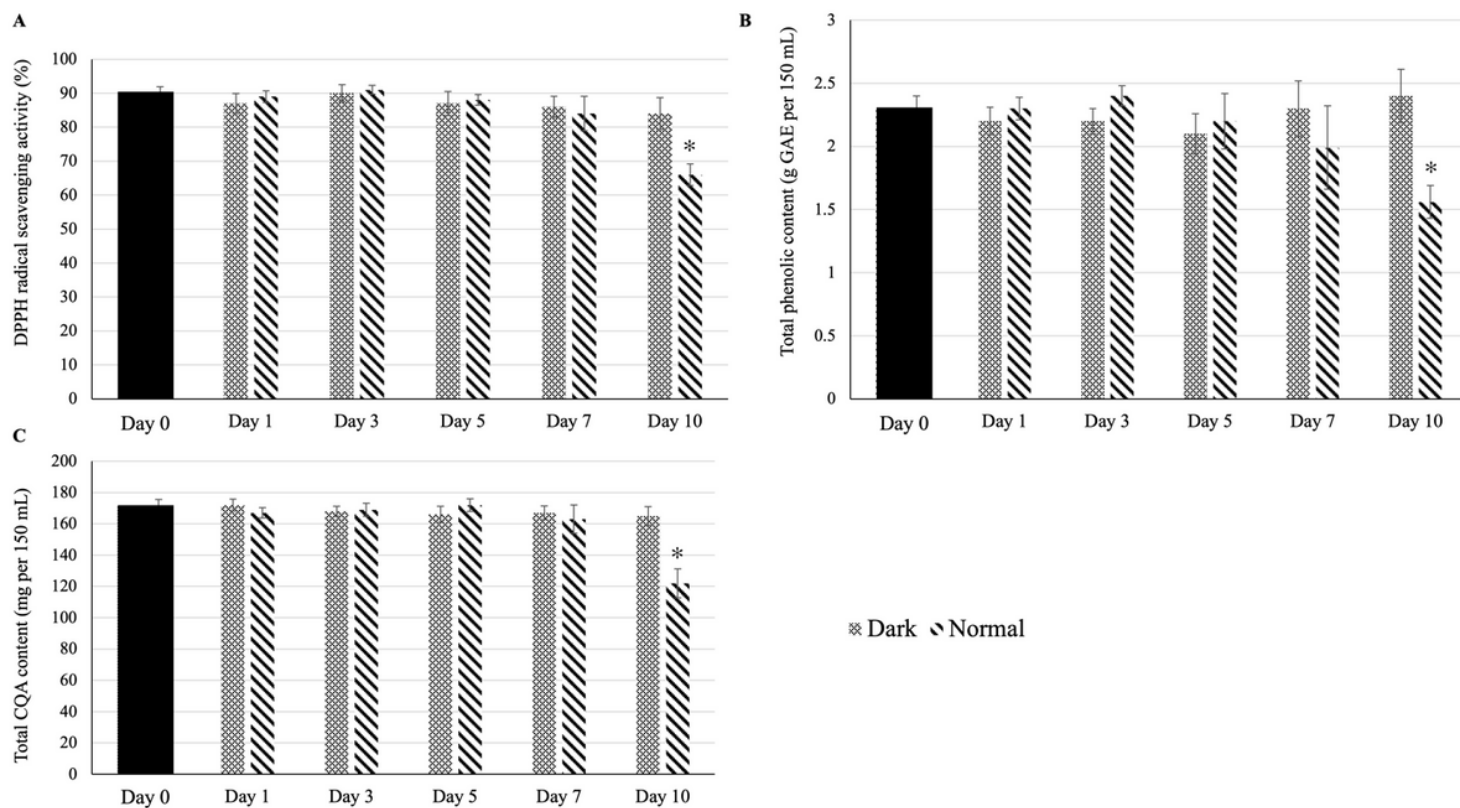


Figure 5

Effect of low-temperature storage on the **(A)** antioxidant activity, **(B)** total phenolic content, and **(C)** total CQA content of yerba mate infusions. Error bars represent means $\pm$ standard deviations ($n = 3$). For each day, an asterisk (*) above the bars indicates a significant difference compared to the sample at day 0 (control) (Student's t -test, $p < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryDatayerbamateMS.docx](#)