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The effect of lemon juice addition and cold storage on the total phenolic content and antioxidant activity of cold mate tea

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ABSTRACT

Although mate tea, made from the infusion of the *Ilex paraguariensis* plant, is typically consumed cold, it should be properly prepared, stored, and consumed within the appropriate time frame to fully appreciate the tea's aroma and components. This study aimed to investigate the impact of adding lemon juice to mate tea and cold storage on the total phenolic content, antioxidant activity, and total mold and yeast count of the tea. It was hypothesized that the addition of lemon juice would increase the total phenolic content and antioxidant activity and extend shelf life. At the start of storage, the sample with 2% (v/v) lemon juice added showed the highest total phenolic content (14.06 ± 0.05 mg GAE/100 mL). The antioxidant scavenging activity of the tea samples was investigated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. While all the studied sample showed higher DPPH inhibition than the control group, the highest value was found for tea sample with 4% lemon addition on 3rd day (97.618 ± 1.429). No mold or yeast growth was observed in the control group and the samples with 2% and 4% lemon juice addition up to day 21. In the sensory evaluation, the tea that contained 4% lemon juice was the most preferred. In conclusion, if mate tea is prepared and stored appropriately, it can maintain its antioxidant capacity for an extended period without the formation of harmful molds or yeasts and any deterioration in its sensory properties.

1. Introduction

Throughout history, people have utilized different parts of plants, such as seeds, leaves, roots, stems, buds, and flowers, to maintain a healthy life (Anand, 2024). Many plant species, through trial and error have been used in the treatment of diseases and also as food, spices, cosmetics, and medicines (Uysal et al., 2023). Medicinal plants contain a variety of bioactive compounds, including flavonoids, anthocyanins, saponins, polysaccharides, alkaloids, carotenoids, organosulphur compounds, terpenes, and phytosterols. These compounds have beneficial effects on health (Martínez-Aledo et al., 2020; Saleem et al., 2022; Shah et al., 2023). In accordance with recent advancements, the use of herbal therapies is being further investigated and is becoming increasingly important for human health due to their mechanisms and duration of action (Aziz et al., 2024). According to the WHO (World Health Organization) Traditional Medicine Strategy 2014–2023, over half of the population use herbal products for the purposes of maintaining good health, preventing, or treating various illnesses (World Health Organization, 2013). The consumption of herbal teas is increasing day by day due to their pleasant taste, aroma, and potential health benefits. Today, the number of plants used for this purpose is quite high (Hosen, 2023). The herbal tea obtained by infusing the dried leaves of the *I. paraguariensis* plant, which is mainly grown in the South of Latin America and is increasingly used all over the world, especially in Western countries, is known as "mate tea" (Bracesco et al., 2011).

It is stated that biologically active phytochemicals, phenolic compounds, xanthines, flavonoids, saponins, amino acids, vitamins, and minerals in the composition of the mate plant are compounds that have positive effects on health (Kaltbach et al., 2020). The growing conditions of the yerba mate plant affect the micronutrient composition of the plant (Magri et al., 2022). In recent years, there has been an increase in the number of studies focusing mainly on the health effects of *I. paraguariensis*. The mate plant has been found to have various pharmacological activities, such as antioxidant, anti-inflammatory, antimutagenic, anti-obesity and cardioprotective (Gómez-Juaristi et al., 2018). In addition, studies have shown that mate can prevent cancer (Jason Saleh et al., 2021), prevent LDL (low-density lipoprotein)

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List of abbreviations

ANOVA	One-way analysis of variance
CFU	cfu, Colony forming unit
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalent
LDL	Low-density lipoprotein
PDA	Potato dextrose agar
SD	Standard deviation
WHO	World Health Organization

oxidation, contribute to the treatment of diabetes and atherosclerosis (Habtemariam, 2019), neuroprotective (Machado et al., 2021), antidiabetic (Valença et al., 2022), antimicrobial (Yuan & Yuk, 2018), vasodilator (Pontilho et al., 2015), hepatoprotective (Hubich et al., 2021) and protective effects against DNA damage (Miranda et al., 2008).

Although tea is typically consumed hot, its consumption as a cold or iced beverage has grown significantly in recent years, particularly during the summer. Even if it is to be consumed as cold or iced tea, to benefit more from its aroma and components, it should be infused with hot water in an appropriate time, brought to room temperature, then cooled in the refrigerator and drunk directly or with ice. No studies have been found in the literature regarding the addition of lemon juice to mate tea, which is also widely consumed cold, or its chemical and sensory changes during the storage period. It was hypothesized that the addition of lemon juice would increase the total phenolic content and antioxidant activity and extend shelf life. In this context, this study; it was planned to examine the effects of adding lemon juice to mate tea to add an additional aroma to the tea, as well as its preparation in large quantities and cold storage under refrigeration conditions on the total mold and yeast count (1), total phenolic content (2), and antioxidant activity (3) of the tea. Also, it was aimed to determine the consumable quality during the addition of lemon juice and the storage period with sensory test (4). In addition to these, pH (5), total acidity (6) and water-soluble dry matter (7) assays were carried out as approximate analyses.

2. Material and methods

2.1. Location, time, sample selection of the research

The analysis of the research was carried out in Izmir Demokrasi University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Food Microbiology and Food Chemistry Laboratory between November 2021 and January 2022.

Mate plant leaf (*I. paraguariensis*) (NCBI taxonomy ID 185542) used within the scope of the research; it was supplied in packaged form (bought from a commercial firm in Türkiye and the firm is declared on the package that origin of mate plant is South America) with the same brand and lot number and the tea was prepared using the infusion method in accordance with the existing literature. Weighings required during the study were made on a 0.01 g sensitive precision balance (Weightlab Instruments WL-3002L, Germany). The lemons used for the study were collected from the garden of the researcher, after being squeezed and filtered, it was added to the tea samples in appropriate ratios without waiting.

2.2. Preparation of mate tea

The packaged mate leaves were stored in a cool and dry place until the tea sample was prepared. The infusion was prepared following the study of Chandra and De Mejia (Chandra & De Mejia, 2004). At the end of the brewing, the infusion was filtered through filter paper (Whatmann, 589/1 black ribbon, 125 mm, Germany) and brought to room temperature. A glass lemon squeezer was used to obtain the lemon juice to be added to the samples other than the control group (sample without lemon juice addition) and the filtrate was passed through filter paper. The obtained lemon juice was added to the tea samples at the ratios of 2% (v/v), 4% (v/v) and 6% (v/v) without waiting. The prepared teas were taken into sterilized 210 mL glass jars and all samples were stored in the refrigerator at +4 °C with their lids closed until the analysis days.

2.3. pH determination

The pH of all tea samples was determined on the 1st, 7th, and 15th days of storage at room temperature with a sensitivity of 0.1 pH meter (AZ Instrument AZ8685, Taiwan). After calibrating the pH meter, the probe was cleaned and immersed in a homogeneous tea infusion, and the pH value displayed on the screen was recorded.

2.4. Determination of total acidity

For the determination of total acidity in all tea samples on the 1st, 7th, and 15th days of storage, 2–3 drops of 1% phenolphthalein indicator (Merck, 107233, CAS 77-09-8) were dripped onto the 25 mL sample. It was then titrated with 0.1 N NaOH solution (Merck, 106498, CAS 1310-73-2) until a light pink color was appeared. The amount of NaOH consumed was recorded in mL and the total acidity was calculated as "g/100 mL" in terms of citric acid (Uylaşer & Başoğlu, 2016).

2.5. Determination of water-soluble dry matter (Brix)

Brix determination of the samples was performed by refractometric method on the 1st, 7th, and 15th days of storage. While the tea samples were at room temperature, reading was made with a hand-held refractometer (RHS-10 ATC, Shenzhen, China) in an environment with sufficient lighting and the amount of water-soluble dry matter was given as "°Brix".

2.6. Determination of total phenolic content

The total phenolic content of mate tea samples was determined by the Folin-Ciocalteu method (Grujic et al., 2012; Suna, 2014).

For the saturated Na_2CO_3 solution used in the analysis, 35 g of anhydrous Na_2CO_3 (Merck, 106392, CAS 497-19-8) was dissolved in 100 mL of distilled water, and a few $Na_2CO_3.10H_2O$ crystals (Sigma-Aldrich, 577782, CAS 6132-02-1) were added after standing overnight. When the crystallization was completed, the content was filtered through glass cotton. Folin-Ciocalteu reagent (Merck, 109001.0500) was purchased as a ready solution.

Tea samples were centrifuged in a laboratory centrifuge (Weightlab Instruments WN-CL4500, Turkey) before analysis. The centrifuged samples were prepared as specified in the method and kept in the dark for 90 min and at the end of 90 min, they were read in a spectrophotometer (Shimadzu UV-1280, Kyoto, Japan) at 720 nm wavelength against the blank.

Each sample was analyzed in three replicates and after calculating the mean absorbance value, the total phenolic content of the samples was calculated as "mg Gallic Acid Equivalent (GAE)/100 g sample" using the standard curve equation $y = 0.2635x - 0.1957$ ($R^2 = 0.9775$) (Uylaşer & Başoğlu, 2016).

2.7. Determination of DPPH-radical scavenger activity

Within the scope of the research, the antioxidant activity of mate tea samples was determined according to the DPPH radical scavenging activity determination method (Bastos et al., 2007; Suna, 2014).

In the analysis adapted from the study of Çarıkçı, DPPH solution (Sigma-Aldrich, CAS 1898-66-4) was added to the sample containing tea at 20, 40, 60, 80, 100 μL concentrations and incubated for 30 min at room temperature in the dark. At the end of the period, the absorbance of the samples was read at 517 nm against the ethanol control and the % inhibition values were calculated (Çarıkçı, 2010).

$$\text{DPPH Activity Inhibition } (\%) = \frac{\text{Acontrol - Asample}}{\text{Acontrol}} \text{ x } 100$$

$A_{control}$ = Absorbance of DPPH solution without sample
A_{sample} = Absorbance of DPPH solution containing sample (Uylaşer & Başoğlu, 2016).

2.8. Mold and yeast count

Microbiological analysis was carried out on the 1st, 3rd, 5th, 7th, 15th, 21st and 28th days. The 1 mL tea sample was added to sterile physiological solution and diluted to serial dilutions. Potato Dextrose Agar (PDA) (Merck, 1.10130.0500) was used as the medium for total mold and yeast counts. The medium was prepared and sterilized according to the instructions on its package. PDA was poured into petri dishes under aseptic conditions and then the prepared dilutions were inoculated by smear plate method. Petri dishes were incubated at 25 °C for 7 days (DH5000BII, Huanghua Faithful Instrument, China) and all colonies were counted at the end of the incubation period. Results were expressed as log cfu/mL. All sterilized materials and chemicals were autoclaved at 121 °C for 15 min (HV50-L, Hirayama, Tokyo, Japan).

2.9. Sensory analysis

A "5-point hedonic scale" was used for the sensory evaluation of cold tea samples containing different concentrations of lemon juice. In the analysis, a group of 15 people, including students and faculty members from various departments of Izmir Democracy University Faculty of Health Sciences, were evaluated. In the sensory evaluation, color, smell, astringency, taste, and general liking parameters were rated on a scale of "1: I did not like it at all, 2: I liked it less, 3: neither like nor dislike, 4: I like it a little bit, 5: I liked it very much". Beforehand, the participants were informed about the form and only individuals who were willing to participate in the study were included in the evaluation. The samples were served to the panelists immediately after being taken out of the refrigerator (4–6 °C), in glasses, each coded with random 3-digit numbers and in random order (Özünlü & Ergezer, 2019). Individuals between the ages of 18–45, without any allergy or intolerance, not taking regular medication, not following a regular diet, not smoking, not hungry or very full at the time of tasting, able to spare time for the evaluation, interested in the topic and volunteers were included in the sensory evaluation (Anonymous, 2012).

2.10. Statistical analyses

The data obtained because of the research are given as mean ± standard deviation in tables. Sensory evaluation data were shown graphically. Statistical evaluation of the data was carried out with the IMB SPSS Statistics 25.0 package program. In this study, the conformity of the data to the assumption of normality; histogram graph, skewness and kurtosis values, coefficient of variation, detrended normal Q-Q plot graph and normality tests were evaluated (George & Mallery, 2019). One-way analysis of variance (ANOVA) was used when the data set showed a normal distribution, and Kruskal-Wallis H test was used when it did not show a normal distribution (Aksakoğlu, 2001). The significance level was accepted as $p = 0.05$. When it was determined that there was a difference between the group means by analysis of variance ($p < 0.05$), multiple comparison (Post Hoc) tests were applied to determine between which groups the difference was. Tukey was used for groups with homogeneous variances, and Tamhane T2 multiple comparison test was used for non-homogeneous groups (Karagöz, 2019).

Table 1
pH values of tea samples.

Samples	1st day	7th day	15th day	Mean ± SD
Control	6.50	6.30	6.50	6.45 ± 0.10
2% lemon juice	3.20	3.20	3.30	3.25 ± 0.05
4% lemon juice	2.80	2.80	2.80	2.80 ± 0.00
6% lemon juice	2.60	2.60	2.60	2.60 ± 0.00

3. Results

3.1. pH, total acidity and water-soluble dry matter

Within the scope of the study, pH values of tea samples by days are shown in Table 1. The pH value varied between 6.30 and 6.60 in the control group and between 3.20 and 3.30 in the sample with 2% lemon juice addition. The pH of the samples with 4% and 6% lemon juice addition did not vary during the storage period. The pH value of the control group was the highest on all days analyzed and it was observed that the pH decreased as the lemon juice addition rate increased.

Total acidity values of tea samples are shown in Table 2 in terms of citric acid. The total acidity of the control group was determined as 0.13 g/100 mL and did not change during the storage period. The total acidity of the group with 2% lemon juice addition rate was between 0.51 and 0.54 g/100 mL, the group with 4% lemon juice addition rate was between 0.92 and 0.97 g/100 mL, and the group with 6% lemon juice addition rate was between 1.35 and 1.41 g/100 mL. It was observed that the total acidity value increased as the lemon juice addition rate increased on all days analyzed.

The water-soluble dry matter content of the tea samples are shown in Table 3 as °Brix. Water-soluble dry matter content was determined as 1.0 °Brix for control samples, 1.5 °Brix for samples with 2% and 4% lemon juice addition and 2.0 °Brix for samples with 6% lemon juice addition. The values did not change in any group during the storage period.

3.2. Total phenolic content

The effect of lemon juice addition and storage time on the total phenolic content of mate tea samples is shown in Table 4 and Fig. 1. On the first day of the analysis, total phenolic content was measured as 13.32 ± 0.00, 14.06 ± 0.05, 13.47 ± 0.00 and 12.83 ± 0.01 mg GAE/100 mL in the control group, 2% lemon juice addition group, 4% lemon juice addition group and 6% lemon juice addition group, respectively. At the end of the storage period, 8.16 ± 0.00, 11.27 ± 0.02, 11.13 ± 0.02 and 11.70 ± 0.01 mg GAE/100 mL were determined in the control, 2% lemon juice, 4% lemon juice and 6% lemon juice groups, respectively.

At the beginning of the storage period, the highest phenolic content

Table 2
Total acidity values of tea samples (g/100 mL)[a].

Samples	1st day	7th day	15th day	Mean ± SD
Control	0.13	0.13	0.13	0.13 ± 0.00
2% lemon juice	0.51	0.51	0.54	0.52 ± 0.02
4% lemon juice	0.92	0.95	0.97	0.95 ± 0.03
6% lemon juice	1.35	1.38	1.41	1.38 ± 0.03

[a] Citric acid.

Table 3
Water-soluble dry matter content of tea samples (°Brix).

Samples	1st day	7th day	15th day	Mean ± SD
Control	1.0	1.0	1.0	1.0 ± 0.0
2% lemon juice	1.5	1.5	1.5	1.5 ± 0.0
4% lemon juice	1.5	1.5	1.5	1.5 ± 0.0
6% lemon juice	2.0	2.0	2.0	2.0 ± 0.0

Table 4
Total phenolic content of tea samples (mg GAE/100 mL).

Days	Control	2% lemon juice	4% lemon juice	6% lemon juice
1st day	13.32 ± 0.00^{aA}	14.06 ± 0.05^{bAC}	13.47 ± 0.00^{cA}	12.83 ± 0.01^{dA}
3rd day	11.76 ± 0.01^{aB}	12.63 ± 0.01^{bB}	11.56 ± 0.02^{cB}	11.61 ± 0.01^{dB}
5th day	12.30 ± 0.01^{aC}	14.01 ± 0.01^{bA}	11.97 ± 0.01^{cC}	10.91 ± 0.01^{dC}
7th day	12.40 ± 0.02^{aD}	13.52 ± 0.01^{bC}	11.93 ± 0.01^{cD}	11.13 ± 0.01^{dD}
10th day	11.77 ± 0.00^{aB}	11.58 ± 0.01^{bD}	11.86 ± 0.01^{cE}	11.05 ± 0.01^{dE}
15th day	11.51 ± 0.02^{aE}	10.31 ± 0.01^{bE}	10.74 ± 0.01^{cF}	10.19 ± 0.01^{dF}
21st day	8.87 ± 0.02^{aF}	11.74 ± 0.01^{bF}	11.18 ± 0.02^{cG}	10.87 ± 0.01^{dG}
28th day	8.16 ± 0.00^{aG}	11.27 ± 0.02^{bG}	11.13 ± 0.02^{cH}	11.70 ± 0.01^{dH}

One-way ANOVA test was used to compare tea samples and days. ^{a,b,c,d} Differences between means marked with different letters in the same row are significant ($p < 0.05$). ^{A,B,C,D,E,F,G,H} Differences between means marked with different letters in the same column are significant ($p < 0.05$).

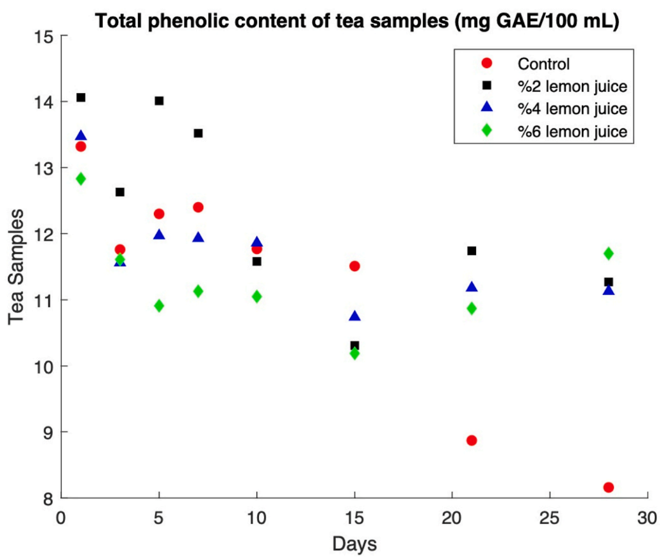

Fig. 1. Total phenolic content of tea samples (mg GAE/100 mL).

was observed in the group with 2% lemon juice addition ($p < 0.05$). Although there was a statistically significant decrease in total phenolic content in all groups during storage period ($p < 0.05$), the greatest decrease was observed in the control group. It was observed that as the rate of lemon juice addition increased, the stability increased and the decrease in the amount of phenolic substances was less.

3.3. DPPH-radical scavenger activity

The percentage inhibition of DPPH free radical of mate tea samples was studied at 5 different concentrations of 20, 40, 60, 80 and 100 μL. The DPPH free radical scavenging activity of the tea samples is given in Table 5 and Fig. 2 as the average of the % inhibition results of the tea concentrations analyzed. On all days of analysis, it was determined that all the lemon juice added samples had higher antioxidant capacity compared to the control sample regardless of the addition rate and the difference was found to be statistically significant ($p < 0.05$). The difference between the samples with the addition of lemon juice was not statistically significant ($p > 0.05$). The change in the antioxidant capacity of the sample with lemon juice addition rate of 2% was not significant during the storage period ($p > 0.05$). Although the decrease in antioxidant capacity from the 3rd day to the 21st day of storage was found to be significant ($p < 0.05$) in samples with 4% and 6% lemon juice addition rates; no statistically significant change was observed from the beginning to the end of storage ($p > 0.05$).

3.4. Total mold and yeast count

The effects of lemon juice addition and storage time on total mold and yeast counts in mate tea samples are shown in Table 6. Within the scope of the study, no mold and yeast growth were observed in the control group without lemon juice addition and in the samples with 2% and 4% lemon juice addition from the beginning of storage to the 21st day of storage. On the 28th day of storage, the control, 2% and 4% lemon juice added samples observed a growth of 3.25 ± 0.06, 3.25 ± 0.94 and 3.60 ± 1.65 log cfu/mL, respectively. Difference between the groups was not significant ($p > 0.05$). With 6% lemon juice addition rate, mold and yeast growth was observed on all days during the storage period, but the difference was not statistically significant ($p > 0.05$).

3.5. Sensory analysis

The results of the sensory evaluation of cold mate tea samples are shown in Fig. 3. When the samples were evaluated in terms of color parameter, the most liked sample was the control sample. This was followed by the samples with 2% and 4% lemon juice respectively. The sample with 6% lemon juice addition received the lowest score in this parameter. For the smell criterion, in contrast to the color, the 6% lemon juice sample was the most preferred sample, and the control sample was the least preferred sample. In the astringency parameter, parallel results were obtained with the smell criterion and the most preferred sample was the sample with 6% lemon juice addition, while the least preferred sample was the control sample without lemon juice addition. When cold mate tea samples were evaluated in terms of taste parameter; the most

Table 5
DPPH-radical scavenging activity of tea samples (% inhibition).

Days	Control	2% lemon juice	4% lemon juice	6% lemon juice
1st day	82.901 ± 2.018^{aABC}	93.140 ± 4.046^{bA}	93.011 ± 3.156^{bAB}	95.255 ± 1.197^{bAB}
3rd day	85.547 ± 1.402^{aAC}	95.406 ± 1.281^{bA}	97.618 ± 1.429^{bA}	97.141 ± 0.879^{bA}
5th day	81.332 ± 1.559^{aAB}	94.330 ± 1.349^{bA}	95.492 ± 1.788^{bAB}	96.598 ± 0.915^{bAB}
7th day	78.367 ± 0.784^{aB}	94.223 ± 1.740^{bA}	96.543 ± 0.718^{bAB}	96.565 ± 0.965^{bAB}
10th day	85.983 ± 0.763^{aC}	93.828 ± 0.948^{bA}	94.178 ± 2.808^{bAB}	95.444 ± 0.688^{bAB}
15th day	89.916 ± 0.812^{aD}	95.098 ± 2.129^{bA}	94.302 ± 2.282^{bAB}	95.265 ± 0.833^{bAB}
21st day	82.554 ± 2.204^{aABC}	94.779 ± 0.916^{bA}	93.337 ± 1.477^{bB}	92.702 ± 1.492^{bB}
28th day	84.678 ± 0.842^{aAC}	96.563 ± 1.017^{bA}	95.057 ± 1.064^{bAB}	94.782 ± 1.907^{bAB}

Kruskal-Wallis H test was used to compare tea samples and days. ^{a,b} Differences between means marked with different letters in the same row are significant ($p < 0.05$). ^{A,B,C,D} Differences between means marked with different letters in the same column are significant ($p < 0.05$).

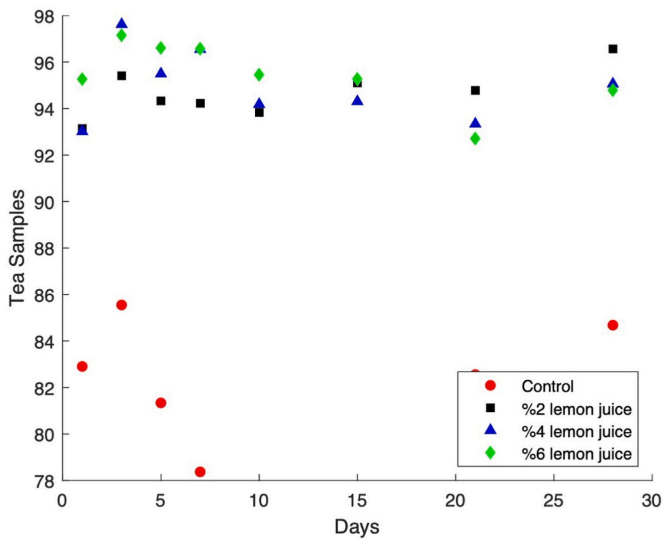

Fig. 2. DPPH-radical scavenging activity of tea samples (% inhibition).

liked sample was the sample with 4% lemon juice addition. Then, 6% lemon juice added sample and 2% lemon juice added sample were preferred respectively. The least liked sample in terms of taste was the control sample, in parallel with the smell and astringency parameters. In the parameter in which the panelists evaluated their general liking about tea by taking all parameters into consideration; the most preferred tea sample was the sample with 4% lemon juice addition as the results were parallel to the taste parameter. The 6% lemon juice sample and the 2% lemon juice sample were then liked. It was determined that the least preferred tea sample by the panelists was the control sample.

Table 6
Total mold and yeast counts of tea samples (log cfu/mL).

Days	Control	2% lemon juice	4% lemon juice	6% lemon juice
1st day	$<1.00^{aA}$	$<1.00^{aA}$	$<1.00^{aA}$	2.28 ± 0.52^{bA}
3rd day	$<1.00^{aA}$	$<1.00^{aA}$	$<1.00^{aA}$	3.31 ± 1.14^{bA}
5th day	$<1.00^{aA}$	$<1.00^{aA}$	$<1.00^{aA}$	3.86 ± 1.20^{bA}
7th day	$<1.00^{aA}$	$<1.00^{aA}$	$<1.00^{aA}$	3.03 ± 1.53^{bA}
15th day	$<1.00^{aA}$	$<1.00^{aA}$	$<1.00^{aA}$	3.60 ± 0.96^{bA}
21st day	$<1.00^{aA}$	$<1.00^{aA}$	$<1.00^{aA}$	5.47 ± 0.08^{bA}
28th day	3.25 ± 0.06^{aB}	3.25 ± 0.94^{aB}	3.60 ± 1.65^{aB}	5.30 ± 0.28^{aA}

Kruskal-Wallis H test was used to compare tea samples and days. [a,b] Differences between means marked with different letters in the same row are significant ($p < 0.05$). [A,B] Differences between means marked with different letters in the same column are significant ($p < 0.05$).

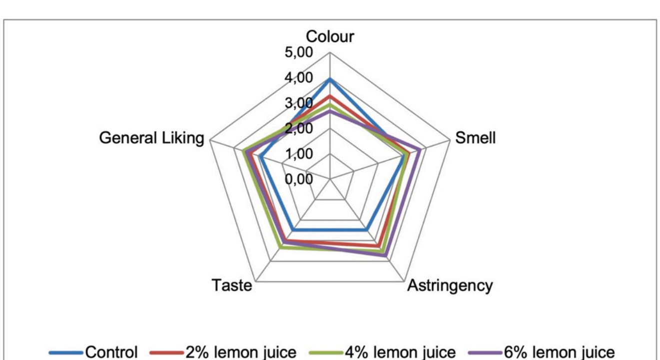

Fig. 3. Sensory evaluation averages of tea samples.

4. Discussion

Our results support the hypothesis that lemon juice addition will increase total phenolic content and antioxidant activity. A significant decrease in the phenolic content of all tea samples was observed during the storage period. With the addition of lemon juice up to 4% by volume, no mold or yeast growth was observed until the 21st day of storage. The hypothesis that the addition of lemon juice will extend the shelf life has been partially confirmed. Sensory evaluation showed that the sample with 4% lemon juice was the most preferred. It is thought that the addition of lemon juice to mate tea, which has an astringent flavor, can be beneficial for health by increasing its consumability and can be consumed as a practical beverage by preparing it in advance and cold storage.

The concept of acidity and pH measurement is a crucial quality criterion in the food and food industry (Tyl & Sadler, 2017), so there are many studies focused on these parameters. In these studies, pH and total acidity of mate tea determined vary but are in similar to with each other (De Oliveira et al., 2017; Pinto et al., 2021; Silva et al., 2022). It is thought that this situation is due to the use of commercial production or tea bags in the studies.

°Brix determination is commonly used to assess the conformity to standards and stability of beverages (Kayısoglu & Coskun, 2021). In a study of conducted by Silva et al., 2022, the water-soluble dry matter content of the commercial mate tea sample was found to be 8.46 °Brix. In our study we measured 1.0 °Brix for the control group. The reason for difference may be resulted mainly the added ingredient and preparation methods of the products.

The relationship between phenolics and antioxidant activity has been reported in many studies, especially high antioxidant capacity of herbal teas is one of the main reasons for tea consumption (Çarıkçı, 2010; Pinto et al., 2021). In studies on the phenolic content of mate tea, it has been reported that alcohol extracts contain higher amounts of phenolic compounds compare to the infusions of tea (Manach et al., 2004; Pinto et al., 2021). In the literature, there are studies showing that the polyphenol levels of extracts obtained from mate plant are higher than green tea and parallel to red wine (Benedito et al., 2023; Crotti et al., 2020; Gugliucci et al., 2009). There are also studies showing that those with high phenolic content have high antioxidant capacity (Baeza et al., 2018). The antioxidant capacity of mate tea was also examined in some different situations such as brewing type and brewing temperature, it was found that the percentage of DPPH radical inhibition ranged from 40.2% to 86.5% depending on the brewing temperature (Janda et al., 2020), and the highest DPPH inhibition rate (92.05 ± 0.60%) was observed in roasted mate extracted with ethanol (Bastos et al., 2007). A study was conducted to investigate the effect of origin and brewing temperature on the antioxidant capacity of tea in yerba mate infusions, traditional mate teas exhibited an average antiradical activity (84.1 ± 2.5%), while non-traditional samples showed an average (78.3 ± 9.9%) (De Mejía et al., 2010). In other study was investigated the various properties of probiotic microorganisms added to mate tea during a 28-day storage period, the total phenolic content of the teas was determined to be 0.50 mg GAE/mL at the beginning of the storage period and 0.51 mg GAE/mL at the end (Silva et al., 2022). Based on the results we obtained, the study findings were found to be lower than the total phenolic substance values reported in the literature. Different values may be attributed to the origin and cultivation of the plants, preparing methods of extracts or unit differences. DPPH inhibition rates results were in similar.

The World Health Organization has set the total limit for microbial contamination in medicinal plants at 10^4/g for plant materials pretreated with boiling water, such as herbal teas, and 10^3/g for other plant materials (World Health Organization, 1998). In Turkey, according to the Turkish Food Codex Regulation on Microbiological Criteria, the limit for the number of yeast and molds in the herbal teas is set as 10^4 cfu/mL (Anonymous, 2011). A study was conducted to assess the microbial load of *I. paraguariensis* leaves, both immediately after harvest and in their processed, packaged form. The mold and yeast count in yerba mate leaves were 3.0×10^4 cfu/g after harvest and 1.5×10^2 cfu/g in packaged form (Albiero et al., 2015). In another study, initially mold and yeast count number the samples of *I. paraguariensis* were found 10^2 cfu/g, then it decreased to <10 cfu/g after infusion (Akduman & Omurtag Korkmaz, 2020). The study's findings are consistent with the existing literature. This study's findings indicate that the addition of lemon juice can enhance microbial stability up to a volume of 4%. However, at a volume of 6%, it creates a favorable environment for mold and yeast growth. It was interpreted that mate tea can be stored up to 21 days with the addition of lemon juice up to 4% by volume and this situation is not microbially risky.

Sensory evaluation of raw materials or products is of great importance for quality control in the food industry. In addition, sensory quality constitutes the quality of food for consumers (Anonymous, 2012). No studies in the literature have researched a sensory evaluation of mate tea using a method like that of the current study. However, a different study conducted by Barboza & Cazal, 2018 nutritional knowledge about mate tea had a positive impact on the acceptability of it.

The main limitation of this study is that the tea samples were stored in separately glass jars based on the lemon juice addition rate and storage time, and the jar lids were not opened until the day of analysis. In mate tea, which is prepared and stored entirely in a single bottle at home, mold and yeast contamination increases as the bottle cap is opened with each consumption, and this may shorten its shelf life. Our second limitation is that all equipment that came into contact with the tea samples was sterilized in an autoclave and worked under aseptic conditions. This condition may have resulted in a longer shelf life for the mate tea than if it had been prepared at home. Another limitation is that the total phenolic content and antioxidant activity of mate tea prepared under home conditions may be lower than that of tea samples prepared under laboratory conditions.

5. Conclusions

It is very important for individuals to turn to natural beverages with high antioxidant capacity, whose effectiveness on health is supported by various studies, instead of sugary and carbonated drinks in the summer months when they need to cool off. Adding lemon juice to mate tea at an appropriate rate can improve its taste by reducing its astringency. Additionally, it is believed that preparing a large quantity and storing it in a cold environment can have health benefits by making it convenient to consume. This study found that following proper preparation and refrigerated storage conditions maintained the product's antioxidant capacity for an extended period. Additionally, no harmful mold or yeast was formed, and there was no sensory deterioration in terms of color, smell, or taste. Mate tea with 4% lemon juice by volume can be recommended as a healthy and refreshing cold drink to individuals that is easy to prepare at home and can be stored in the refrigerator for up to 21 days (by keeping the tea preparation and storage method like laboratory conditions). Despite the numerous studies conducted on herbal teas, including mate tea, no literature has been found on the addition of natural substances to reduce tea's astringency, increase its consumption, or enhance its flavor. In further studies, aroma and main phenolic components of tea samples can be determined by using various spectroscopic and chromatographic methods. Additionally, the nutritional value and content changes during the shelf life of tea samples can be examined.

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CRediT authorship contribution statement

Nedime Gündüz: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Reyhan Irkin:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization. **Sema Çarıkçı:** Writing – review & editing, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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