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Investigation about Various Infusion Conditions on Physical, Chemical and Antioxidant Properties of *Clitoria ternatea* L. Tea

Melek ZOR[1], Memnune ŞENGÜL[2], İsa Arslan KARAKÜTÜK[2*], Sefa AKSOY[2]

Highlights:

- The infusion temperature, infusion time and particle size of *C. ternatea* teas have led to significant changes in changes in the physical and chemical properties of the teas
- Antioxidant activity of teas increased with increased infusion temperature and infusion time
- The antioxidant activity of teas infused with flower powder is higher than teas infused with whole flowers

Keywords:

- *Clitoria ternatea* L.
- Infusion temperature
- Infusion time
- Particle size
- Antioxidant activity

ABSTRACT:

The study detected changes in some physical, chemical, and antioxidant properties of *Clitoria ternatea* L. teas infused at different infusion temperatures (ITE) (70 °C, 80 °C, and 90 °C) and infusion times (ITI) (9, 18, and 27 minutes) using dried flowers of particle sizes (PS) (whole and powder). The antioxidant activity was measured using DPPH, ABTS, and FRAP methods. The a*, b*, C*, and Hº values of the tea samples were found to be statistically different according to ITE, ITI, and PS (p<0.01). A significant decrease was identified in averaged a*, b*, and C* values with increasing ITE. There was a significant decrease in mean L* values of tea samples with increasing ITE (p<0.05). The total monomeric anthocyanin (TMA), total phenolic content (TPC), and antioxidant activity of tea samples differed significantly with ITE, ITI, and PS (p< 0.01). The highest TMA and TPC according to ITE (69.72 Cy-3 glu mg/L and 1000.68 mg GAE/L) in tea samples were at 90 ºC and the highest TMA and TPC according to ITI (51.54 Cy-3 glu mg/L and 918.45 mg GAE/L) were detected at 27 minutes of infused. It was determined that the TMA of the teas infused with whole flowers was higher (55.31 Cy-3 glu mg/L) than the teas infused with flower powder, and the TPC was higher (926.07 mg GAE/L) in the teas infused with the flower powder. The total flavonoid content (TFC) of the teas was determined the highest (5161.69 mg QE/L) according to ITE at 80 ºC and the highest TFC (4578.53 mg QE/L) according to ITI in 9 minutes of infused. It was observed that antioxidant activity of tea samples increased with increasing ITE and ITI. Regarding PS, it was also determined that teas brewed with flower powder showed higher antioxidant activity. According to the sensory evaluation results of tea samples, it was found that tea samples infused with whole flowers at 70 ºC for 9 minutes and tea samples infused with flower powder at 80 ºC for 27 minutes had the highest overall acceptability.

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INTRODUCTION

Clitoria ternatea L., which is also known as butterfly pea, blue pea flower, or blue butterfly pea and which is native to South Asia and Southeast Asia, is a perennial plant belonging to the Fabaceae family (Jeyaraj et al., 2021; Ramli et al., 2021; Permatasari et al., 2022). In other regions, it is known as lan hu die (Chinese), aparajita (Bengali), kajroti (India), cunha (Brazilian), clitoria azul (Spanish), cunhã, fula criqua (Portuguese), bunga biru, tembang telang (Indonesian), chi dậu biếc (Vietnamese), bunga telang (Malaysian), dangchan (Thai), and mavi kelebek sarmaşığı (Turkish) (Jeyaraj et al., 2021). The C. *ternatea* L. flowers are used as tea, food, for decorative purposes and as natural dyeing agents. *C. ternatea* L. has allergy, cough, arthritic, neuroprotective, anti-depressant, anxiolytic, sedative, anti-convulsant, hepatoprotective, anti-inflammatory, antidiabetic, and anticancer properties and the potential to cope with other life-threatening diseases. The mentioned prophylactic activities originate from varying contents of polyphenolic compounds (polyphenols, flavonols, flavonoids, anthocyanins, etc.) (Mehmood et al., 2019; Kumari et al., 2021). The unique property of anthocyanins in *C. ternatea* flowers results from abundant polyacylated anthocyanins known as "ternatins" (Netravati et al., 2022).

The aqueous extract of *C. ternatea* flowers is traditionally utilized in Asia as a natural colorant in food and beverages. In Malaysia, its aqueous extract is utilized for the purpose of coloring rice cake and the popular dish “Nasi Kerabu” and for its therapeutic properties. Moreover, it is used in traditional Indian and Ayurvedic traditional medicine to relieve constipation, indigestion, arthritis, skin diseases, and intestinal and liver problems (Escher et al., 2020a; Jeyaraj et al., 2021). Furthermore, research has demonstrated that the aqueous extract of *C. ternatea* does not exhibit cytotoxicity in human fibroblast cells, has a protective impact on human erythrocytes, and inhibits the oxidation of plasmid DNA, maintaining its toxicological safety and bioactivity (Mehmood et al., 2019; Escher et al., 2020b).

In this respect, the colorant obtained from the *C. ternatea* flower is the first FDA-approved plant-based natural source of blue color in the industry. This natural colorant fills a significant gap in the natural color spectrum, particularly due to its exceptional heat and light stability. The water-soluble colorant ensures a bright blue denim hue in products with a pH value higher than 3.8 while providing a unique deep purple hue in products with a lower pH value, such as sports drinks (Adams, 2022). Using this property, the pigment acquired from the *C. ternatea* flower was used as a pH indicator in acid-base titration (Campbell et al., 2019). Moreover, it has been reported that, blue butterfly flower anthocyanins can be used with hydroxypropyl methylcellulose/microcrystalline cellulose biocomposites in smart food packaging design using their color change at different pH values (Boonsiriwit et al., 2021).

Apart from these usage areas, it is important to determine the most appropriate infusion conditions in consuming blue butterfly flowers as tea in order to meet the health impacts we mentioned in the best way. The infusion procedure applied while obtaining herbal teas is provided by keeping plant materials in a continuously boiling water for different periods of time or in hot water at different temperatures (Kılıç et al., 2017). Infusion conditions are also of great importance to consumers, as the beneficial effects of tea are related to the amount of bioactive compounds that pass into the water from the plant material (Fibrianto & Kinsky, 2020). The transition amount of these compounds may depend on the amount of plant materials used, the particle size, the amount and temperature of the water used in infusion, the infusion time, whether there is mixing process, and the addition of ingredients such as sugar or milk (da Silveira et al., 2014). In this study, it was aimed to determine the physicochemical properties and the changes in total phenolic content, total monomeric anthocyanin content, total flavonoid content and antioxidant properties of tea infused at different temperatures and different times from all blue butterfly flowers and powder forms, and to determine the optimum conditions in terms of these

properties in infusion. In addition, the effects of teas on consumer preferences were determined by sensory evaluation.

MATERIALS AND METHODS

Materials

Clitoria ternatea flowers grown in Turkey in 2022 were purchased in a dried form from a local market in Erzurum province. Figure 1 presents the images of *Clitoria ternatea* flowers (CTF) and *Clitoria ternatea* flower powder (CTFP) used in the study. The preparation of plant infusions and analyses were conducted at Atatürk University, Faculty of Agriculture, Department of Food Engineering Laboratories.

Figure 1. A) *Clitoria ternatea* flowers *B) Clitoria ternatea* flowers powder

Methods

Preparation Of Blue Butterfly Flower Teas

The flowers were cleaned by hand and turned into powder using a laboratory-type blender (Waring HGB2WTS3, USA). CTF and CTFP were sealed in plastic bags and stored at -20 ℃ for further analysis. The brewing process was done by making modifications to the method of Topdaş (2022).

Table 1. Infusion conditions of *Clitoria ternatea* flowers and powder

	Infusion conditions	
	Temperature (°C)	**Time (m)**
***Clitoria ternatea* flowers (CTF)**	70	9
		18
		27
	80	9
		18
		27
	90	9
		18
		27
***Clitoria ternatea* flower powder (CTFP)**	70	9
		18
		27
	80	9
		18
		27
	90	9
		18
		27

The infusion procedure was carried out according to the conditions specified in the Table 1 by the infusion method in a water bath by taking 3 g of whole and powdered dried flowers and adding 100 mL of water previously brought to the appropriate temperature for each infusion temperature. After the infusion times, the samples were filtered through filter paper (Whatman No:1), and the filtrates were utilized in the analysis. All extraction procedures and analyses were conducted in tea infusions.

Determination Of Color Values (L*, a*, b*, C*, and Hº)

The tea infusions color intensities were determined with a colorimeter (Konica Minolta CR-400, Korea) performing three-dimensional measurements in the CIE (L*, a*, b*, C*, H°) system. The standard white plate of the device was used for the calibration of the colorimeter. The color values for each sample were read on a white background at 20±2 °C. L*, a*, b*, C*(Chroma, color saturation), and H° (Hue angle) values were determined (Wrolstad et al., 2005; Zor & Şengül, 2022; Acar et al., 2022). Chroma and hue angle values were calculated using the following equation 1-2.

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$H^\circ = tan^{-1}(b^*/a^*) \quad (2)$$

pH And Titration Acidity Analysis

The tea infusions pH values were measured at room temperature (20±2 °C) with a digital pH meter (Ohaus, starter 3100, ABD) (Cemeroğlu, 2013). The titration acidity of samples amounts were determined by electrometric titration with 0.1 N NaOH solution up to pH 8.1 (Cemeroğlu, 2013). The results are given in g citric acid (CA)/100 mL.

Total Monomeric Anthocyanin Content

Total monomeric anthocyanin (TMA) content in the tea samples was determined spectrophotometrically by employing pH differential method, and the results are presented as mg/L in terms of cyanidin-3-glucoside (Cy-3 glu) (Cemeroğlu, 2013). Tea treatments were diluted with buffers (pH 1.0 and 4.5) by utilizing potassium chloride (0.025 M) and sodium acetate (0.40 M), respectively, by employing the previously determined dilution factor. Afterward, the dilutions were allowed to equilibrate for 30 min. The absorbance of every sample solution was recorded with a spectrophotometer (PG Instruments T60V, UK) calibrated with distilled water as the blank at wavelengths of 515 and 700 nm. The following formula was used to calculate the difference in absorbance between pH values (1.0 and 4.5) and wavelengths:

$$A = (A_{515} - A_{700})_{pH1.0} - (A_{515} - A_{700})_{pH4.5} \quad (3)$$

The TMA in the sample was computed as cyanidin-3-glucoside with the equation below:

$$TMA\ (mg/L) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (4)$$

Where MW; refers to the molecular weight (449.2 g/mol for cyanidin-3-glucoside), DF; denotes the dilution factor, ε; represents the molar absorbtivity (26900 for cyanidin-3-glucoside), and l refers to the path length (1 cm).

Total Phenolic Content

Extracts were prepared by modifying the method described in the study by Ciniviz and Yildiz (2020). Three mL of the sample was mixed with 30 mL of methanol and subjected to extraction in an ultrasonic water bath at 30 ºC for a period of 30 minutes. Then, it was centrifuged at 6000 rpm at 4 ºC for a period of 15 min. The filtrate was filtered through Whatman No. 42 filter paper. The acquired

extracts were used in the total phenolic content **(**TPC), total monomeric anthocyanin (TMA), total flavonoid content (TFC), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS), and ferric (III) reducing ability of plasma **(**FRAP) analyses.

TPC was identified with the Folin-Ciocalteu reagent (FCR) following the method described by Meda et al. (2005). First, 100 µL of the sample was pipetted into glass tubes, then 2.5 mL of 0.2 N FCR was added, and the tube was vortexed. After 3 minutes, 2 mL of 7.5% Na_2CO_3 was added, and the tubes were vortexed again. After incubating the samples for 2 hours at room temperature in the dark. The absorbances of the samples and blank solutions containing all other chemicals without samples were read at 760 nm on a UV-visible spectrophotometer (PG InstrumentsT60V, UK). To determine the total phenolic content, calculations were made with the help of a calibration curve (r^2=0.991) using the gallic acid standard consisting of different concentrations. The results are expressed as mg gallic acid equivalent (GAE)/L sample.

Total Flavonoid Content

The total flavonoid content in tea samples was determined spectrophotometrically in line with the method proposed by Koçak et al. (2018). 0.25 mL of the tea samples extracts were taken, and 1.25 mL of distilled water was added to them. Afterward, 0.075 mL of 0.05 g/mL $NaNO_2$ was added and kept for 6 minutes by vortexing. Then, 0.15 mL of 0.1g/mL $AlCl_3 \cdot 6H_2O$ was added, vortexed, and kept for 5 minutes. Finally, 0.5 mL of 1 mol/L NaOH was added, mixed by vortexing, and incubated for 15 minutes. At the end of the incubation period, the sample absorbances were read at a wavelength of 510 nm. To calculate the total flavonoid content, 10-250 mg/L quercetin was prepared, and a calibration curve was drawn. Thus, the total flavonoid content was computed as mg quercetin equivalent (QE)/L sample with the equation acquired from this calibration curve.

Antioxidant Activity

DPPH Radical Scavenging Activity

The tea infusions DPPH radical scavenging activity was determined according to the study by Gülçin (2005). Amounts were taken from the extracts at different concentrations (10-30 µL/mL) and made up to 2 mL with ethanol. Then 500 µL of DPPH solution was added to the samples. The test tubes mixed by vortexing were left to incubate in a dark environment and at room temperature for 30 minutes. The samples' absorbances were measured in a spectrophotometer at a wavelength of 517 nm. Using the absorbances read in the measurement results, % inhibition values were computed with the help of the equation presented below (Equation 5).

$$\%\, Inhibition = [(A_{DPPH\cdot} - A_{sample})/A_{DPPH\cdot}] \times 100 \quad (5)$$

Here;

A_{DPPH}: Absorbance of the DPPH blank sample (nm)

A_{sample}: Absorbance of the sample extract (nm)

The tea samples IC_{50} values (the concentration inhibiting 50% of the radical) were calculated. While calculating this value, sample inhibition values were plotted against sample volumes to reach the curve and equation needed, and linear regression analysis was applied. As a result of this analysis, the IC_{50} values of samples were computed using the equation of the curve related to the sample (Zor et al., 2022).

ABTS Radical Scavenging Activity

The ABTS radical scavenging activity of tea samples was determined according to the study by Köksal et al. (2009). While preparing ABTS radicals, they were obtained by adding 2.45 nM potassium persulfate solution to ABTS solution prepared at a concentration of 2 mM with distilled water and mixing at room temperature and in the dark environment for 16 hours. Prior to the analysis, the absorbance of ABTS solution was diluted to 700±25 absorbance at 734 nm. The extracts to be analyzed were transferred to test tubes to form a concentration of 10-50 µL/mL. Afterward, the total volume was made up to 2 mL with ABTS solution, and each tube was vortexed and incubated for 6 minutes at room temperature and in the dark. At the end of the incubation period, the samples' absorbances were read against a blank at a wavelength of 734 nm. The control consists of the ABTS solution. The lightening of the color in the sample solutions indicates the antioxidant effect, and the % inhibition values of the samples in this analysis at different concentrations were computed using the equation 6 below.

$$ABTS^{+\cdot}\ Inhibition\ (\%) = [(A_{ABTS} - A_{sample})/A_{ABTS}] \times 100 \quad (6)$$

A_{ABTS}= Absorbance of $ABTS^{+\cdot}$solution (nm)

A_{sample} = Absorbance of the sample (nm)

Linear regression analysis was applied to the sample volumes plotted against the samples' inhibition values. The curve and equality values of the sample were obtained as a result of this analysis. The samples' IC_{50} values were calculated using the equation acquired (Zor et al., 2022).

Ferric (III) Reducing Ability Of Plasma (FRAP)

It is based on reducing Fe^{3+} ions in the Fe $(TPTZ)^{3+}$ mixture present in the radical to be used in determining the antioxidant activity of tea samples by the FRAP method to the blue-colored Fe $(TPTZ)^{2+}$ complex in acidic medium (Koçak et al., 2018). Antioxidant activity was determined by the FRAP method by making some modifications to the method suggested by Koçak et al. (2018). The solvents used in the study were prepared daily as three solutions;

1-Using acetate buffer (pH 3.6) as 3.1 g sodium acetate+16 mL acetic acid in 1 L solution,

2-By dissolving 0.156 g of TPTZ (2,4,6-tripydryl-s-triazine) in 50 mL of ethanol and

3-As 0.5404 g $FeCl_3 \cdot 6H_2O$+2 mL HCl (37% m/m) in 100 mL solution.

The solutions were mixed at a ratio of (10:1:1), respectively, and thus, the FRAP reagent was prepared. 900 µL of the FRAP reagent was added to 100 µL of the sample extract, and the mixture was vortexed. Absorbance was measured at 593 nm after 4 minutes. A calibration curve was acquired using Trolox consisting of different concentrations (5-25 µM). The results were calculated in µM TE/mL using this calibration curve.

Sensory Evaluation

The sensory properties of tea infusions were identified according to the method suggested by Zhang et al. (2021). Twenty-five panelists who were experienced and familiar with tea evaluated the sensory properties of the tea samples. The tests were carried out by students and lecturers of Atatürk University Food Engineering Department (Erzurum, Turkey) at the Food Engineering Department of Atatürk University. Tea samples were presented in random order, identified with random three-digit codes at a service temperature of 45±5 °C. Tea samples (approximately 30 mL) were placed in special transparent glasses. Each panelist evaluated the tea samples in terms of six sensory properties: gloss/opacity, infusion color, astringency, flavor, mouthfeel, and general acceptability. All sensory attributes were recorded on scales from 1 (poor) to 9 (excellent), and the final results were interpreted according to the mean score of each parameter.

Statistical Analysis

The data obtained in triplicate were analyzed using the SPSS 20.0 program. The results were presented as standard deviation (±SD) and mean values. 3-way analysis of variance (ANOVA) and pearson's correlation test were conducted with the objective of determining the significant group differences between the means ($p \leq 0.05$, $p \leq 0.01$). Duncan's multiple range test was carried out for comparing mean values. Moreover, principal component analysis (PCA) was applied to some data to facilitate the identification of similarities and differences between the samples (SIMCA-P + 14.1, UMETRICS).

RESULTS AND DISCUSSION

Table 2 shows the color values (L*, a*, b*, C*, and Hº) of tea infusions, which were prepared by infusing the whole and powdered *C. ternatea* flower at different temperatures and times, and changes in pH and titration acidity. It was determined that, a significant decrease occurred in the mean L* values with the increasing infusion temperature in the tea samples ($p<0.05$), and therefore, the increase in the infusion temperature caused the darkening of the color (Table 2). The a*, b*, C*, and Hº values of the tea samples were found to differ statistically according to infusion temperatures (ITE), infusion time (ITI), and particle size (PS) ($p<0.01$). A significant decrease was identified in the L*, a*, b*, and C* values with the increase in the infusion temperature. However, it was revealed that, this decrease was higher at 80 ºC than at 90 ºC. It was also determined that, the H° angle value decreased at 90 ºC and increased at 80 ºC compared to the values at 70 ºC (Table 2). The increase in the infusion time resulted in a significant decrease in the L*, a*, b*, C*, and Hº values in the samples ($p<0.05$). The decrease in the L* value indicates the darkening of the color, the decrease in the a* value indicates the decrease in redness, and the decrease in the b* value shows the shift of the color toward yellow. A study carried out by Topdas (2022) with different herbal teas reported that the increase in infusion time reduced the L* value, increased the a* value, and decreased the b* value of samples in general. In addition, Liu et al., (2018) reported that L* and a* values decreased, while b* values increased with the increase of infusion time in green tea samples.

Particle size (PS) became statistically effective on the color values of infused teas at a $p< 0.01$ level, and the L*, a*, b*, C*, and Hº values of teas prepared from CTFP were determined to be lower than those prepared from CTF. Based on these results, it can be stated that teas prepared from CTF have a darker color than teas prepared from CTFP, have higher redness, and exhibit a characteristic closer to blue color.

Statistically significant changes were observed in the pH value and the amount of titration acidity of teas according to the infusion temperature and particle size ($p<0.01$). The pH values increased with the increasing infusion temperature, and the highest mean value was determined at 90 ºC. A decrease was observed in titration values with the increasing temperature (Table 2). Dinçer (2022) reported that an increase in infusion temperature caused an increase in pH values in acacia and squash blossom teas, and a decrease in honeysuckle, lilac, clove, and bud flower teas. In the same study, it was reported that the increase in the infusion temperature caused a decrease in the titration acidity values of the pumpkin flower teas, and the increase in the infusion temperature caused an increase in the titration acidity values of the other teas in the study. In other words, it was reported that differences were observed in pH and titration acidity values according to the applied temperature and flower variety. While no statistically significant change was observed in pH values according to infusion time ($p>0.05$), the effect of infusion time on titration acidity values was found to be significant ($p<0.01$) (Table 2). Liu et al., (2018)

determined that with the increase of infusion time, there is a decrease in the pH value of the medium and an increase in the amount of titration acidity. The study by Dinçer (2022) reported that only the pH value of the pumpkin flower tea increased with the increasing infusion time, whereas the values of other teas decreased. The same study indicated an increase in the amount of titration acidity of all flower teas (a decrease after an increase in honeysuckle, pumpkin, and clover flower teas) together with the increasing infusion time applied during the preparation of flower teas.

Double and triple interactions of infusion temperature, infusion time, and particle size factors were found to be statistically significant on L*, a*, b*, C*, H°, pH and titration acidity amounts of CTF and CTFP teas (p<0.01).

Table 2. Changes in some physicochemical properties of CTF and CTFP teas according to infusion temperature, infusion time and particle size factors

	L*	a*	b*	C*	H°	pH	Titration acidity (g CA/100 mL)
Infusion temperature (ITE)							
70 °C	38.27±1.20^{a}	4.00±1.51^{a}	4.26±1.82^{a}	5.88±2.28^{a}	46.23±6.60^{b}	6.23±0.09^{c}	0.019±0.00^{a}
80 °C	37.45±0.58^{c}	2.45±0.80^{c}	2.84±0.81^{c}	3.78±1.04^{c}	49.37±7.18^{a}	6.31±0.07^{b}	0.019±0.00^{a}
90 °C	37.72±0.60^{b}	3.46±0.45^{b}	3.22±0.84^{b}	4.74±0.89^{b}	42.37±4.04^{c}	6.36±0.02^{a}	0.018±0.00^{b}
Significance	*	**	**	**	**	**	**
Infusion time (ITİ)							
9 Minute	38.12±1.00^{a}	3.74±1.62^{a}	3.95±1.52^{a}	5.47±2.13^{a}	47.47±7.55^{a}	6.30±0.09	0.019±0.00^{b}
18 Minute	37.84±0.98^{b}	3.27±1.04^{b}	3.45±1.51^{b}	4.77±1.77^{b}	45.72±6.26^{b}	6.31±0.07	0.019±0.00^{b}
27 Minute	37.49±0.58^{c}	2.91±0.61^{c}	2.92±0.79^{c}	4.15±0.90^{c}	44.78±6.12^{c}	6.30±0.11	0.020±0.00^{a}
Significance	**	**	**	**	**	ns	**
Particle size (PS)							
Clitoria ternatea flowers (CTF)	38.21±0.97^{a}	3.41±1.37^{a}	4.03±1.50^{a}	5.30±1.96^{a}	50.22±6.38^{a}	6.25±0.09^{b}	0.018±0.01^{b}
Clitoria ternatea flower powder (CTFP)	37.41±0.61^{b}	3.20±1.00^{b}	2.85±0.92^{b}	4.30±1.34^{b}	41.76±3.46^{b}	6.35±0.05^{a}	0.020±0.02^{a}
Significance	**	**	**	**	**	**	**
ITE X ITİ	**	**	**	**	**	**	**
ITE X PS	**	**	**	**	**	**	**
ITİ X PS	**	**	**	**	**	**	**
ITE X ITİ X PS	**	**	**	**	**	**	**

a-c: means with different letters in the same column are significantly different (p< 0.05); ns: not significant (p> 0.05); * p< 0.05; ** p< 0.01. Raw data not shown.

Table 3 demonstrates changes in total monomeric anthocyanin content, total phenolic content, total flavonoid content, and antioxidant activity of CTF and CTFP infusions obtained at different temperatures and times. Infusion temperature and infusion time had significant effects on the TMA, TPC, and TFC of tea samples (p< 0.01) (Table 3). Increasing the infusion temperature and infusion time of the tea samples increased the TMA and TPC values, while decreasing the TFC values (Figure 2). In line with our results, Acar et al., (2022) reported that higher phenolic content was detected in teas (made with food waste) infused at 70 °C, except for one of the teas (made with food waste) infused at 100 °C. It was indicated that this situation might be related to the fact that the increasing infusion temperature increased the interaction of substances in contact with the solvent, facilitating the migration of polyphenols into water (Cacace and Mazza, 2003). Kılıç et al. (2017) and Acar et al. (2022) reported

that TPC increased with the increasing infusion temperature in the infusion of herbal (corn tassel, walnut shell, cherry stalk, banana peel, pomegranate peel, mandarin peel, eggplant peel and red onion peel) teas.

According to the infusion time, the highest TMA and TPC were identified at the 27-minute infusion time (Table 3). Likewise, a recent study reported that an increase in the infusion time at a constant temperature increased TPC and antioxidant activities (Gan and Ting, 2019). In addition, in a study, it was reported that the increase in infusion time in teas infused from different plant wastes caused a decrease or increase in TPC according to the plant waste type (Acar et al., 2022). In the literature review, it is seen that the effect of infusion time on TPC in herbal teas differs according to the plant material. On the other hand, Polat et al. (2022) stated that the total polyphenol content in black tea infusions changed depending on the infusion time, achieved the highest level at 30 minutes and then decreased, and high temperature and long infusion time could lead to degradation of phenolic compounds and complex formation between minerals and phenolic compounds.

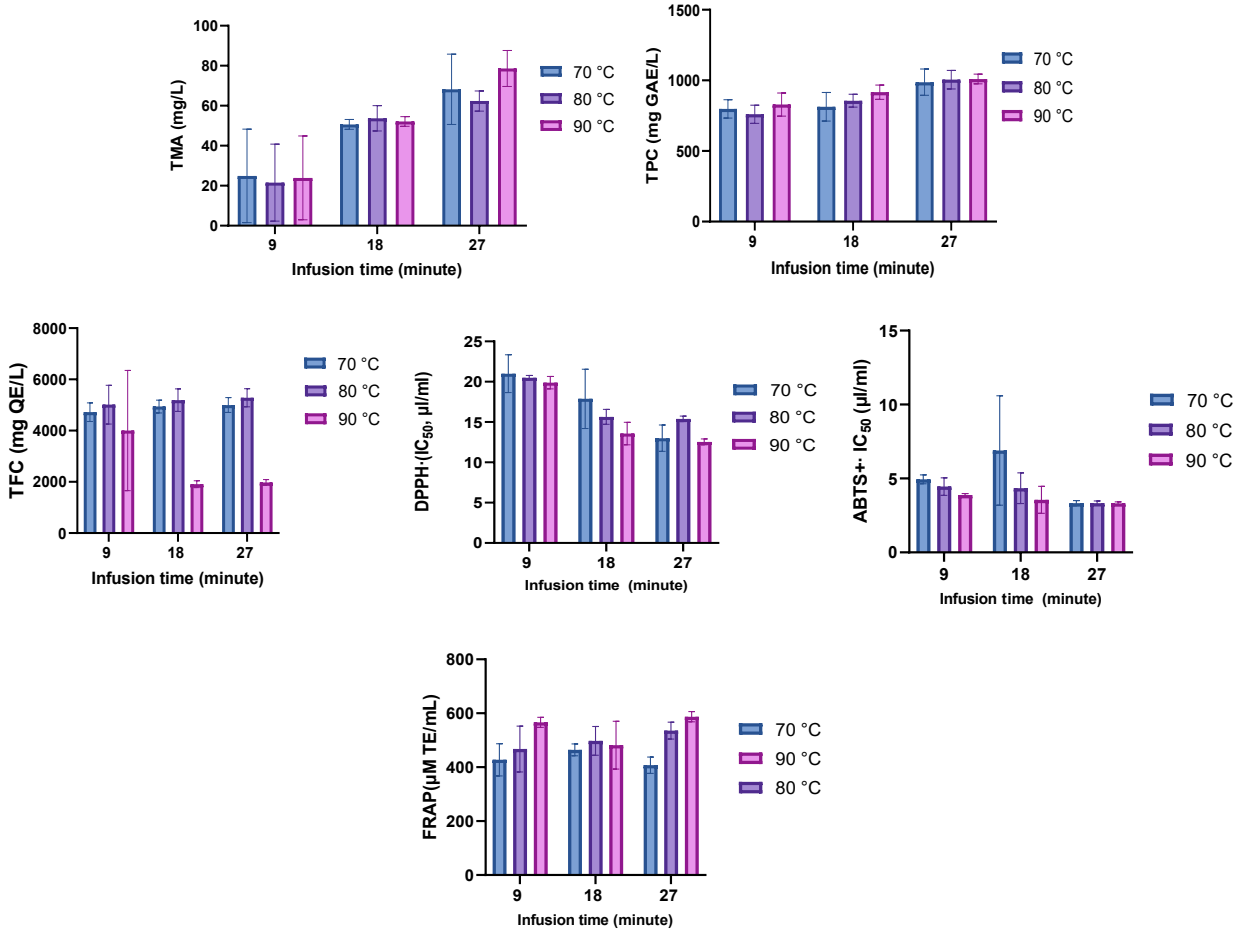

Figure 2. TMA, TPC, TFC, and antioxidant properties of teas according to infusion time × infusion temperature

As shown in numerous studies, the butterfly pea flower is among the most important sources of polyacylated blue-colored anthocyanins. Polyacylated anthocyanins are highly soluble in water and have been demonstrated to have significantly higher stability, especially under low-acid and neutral conditions (Netravati et al., 2022). It is known that the stability of anthocyanins with bioactive properties is impacted by a number of factors, including temperature, pH, light, chemical structure, presence of oxygen, solvent, presence of accessory pigments, enzymes, and metal ions (Giusti & Wrolstad, 2003).

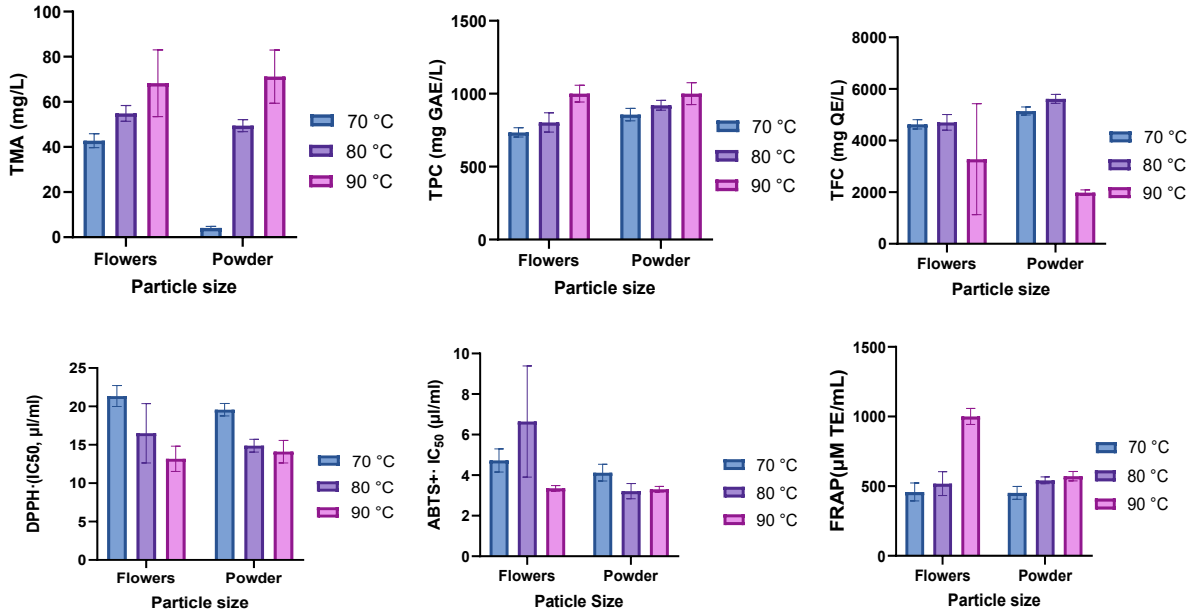

Figure 3. TMA, TPC, TFC, and antioxidant properties of teas according to particle size × infusion temperature

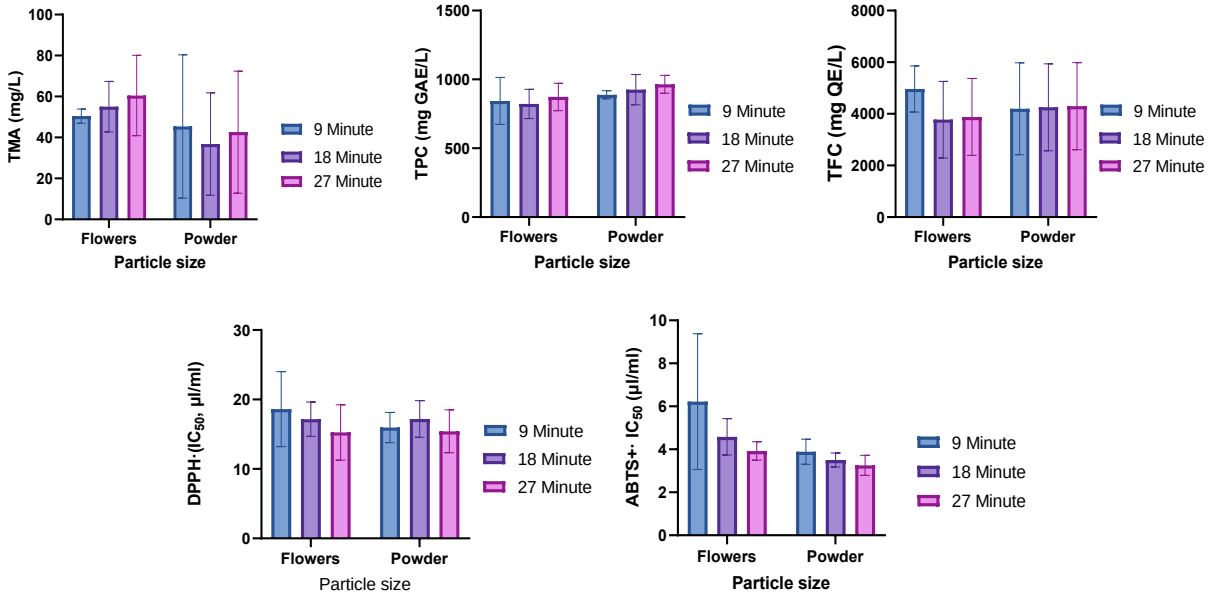

Figure 4. TMA, TPC, TFC, and antioxidant properties of teas according to particle size × infusion time

Thuy et al. (2021) argued that 45 °C and 60 minutes were the optimum extraction temperature and time for anthocyanin extraction and reported that anthocyanins could not be completely dissolved in the solvent when the time was too short or too long in anthocyanin extraction, high total anthocyanin content could not be acquired if the time was too short, and in addition to all these, they would degrade if they were extracted for a longer time at high temperatures since they were heat sensitive pigments. According to Patras et al. (2010), temperatures higher than 50 °C during processing caused partial or complete degradation of anthocyanins and a decrease in color intensity. However, Jeyaraj et al. (2021) reported that the extraction temperature for anthocyanin extraction increased with the increasing temperature and the optimum temperature was 70 °C, and the increase in the extraction temperature resulted in higher extraction of anthocyanins since it increased the internal energy of molecules increasing the diffusion and solubility of pigments and thus having a higher yield. On the other hand, Ludin et al. (2018) indicated

a decrease in anthocyanin concentration at 80 °C, which might have been caused by the degradation of pigments. Particle size had a significant effect on TMA and TPC ($p< 0.01$), but particle size did not cause a significant change for TFC ($p> 0.05$). Upon evaluating the TFC results of teas, the highest contents were determined at 80 °C, and the lowest contents were found at 90 °C. It was observed that a 9-minute infusion time yielded higher TFC results in teas than other infusion times (Table 3). It was determined that the TMA contents were higher in the tea samples infused with CTF compared to the teas infused with CTFP, whereas the TPC was revealed to be higher in the teas infused with CTFP compared to the samples infused with CTF (Figure 4).

It was observed that, antioxidant activity increased in the antioxidant activity assays (DPPH, ABTS, and FRAP) with the increasing infusion temperatures and infusion times of tea samples (Table 3), which is thought to be caused by the bioactive components becoming freer as a result of further degradation of tissues in the plant material under the impact of increasing temperature and time. Based on the results, a direct relationship is observed between TPC and antioxidant activity (Figures 2-3). In their study, Chang et al. (2020) researched the impact of infusion in water at 60, 70, 80, 90, and 100 °C for 1 minute on the total phenolic content and antioxidant activity in black tea and reported that black tea displayed the increased antioxidant activity when the infusion temperature was increased. Therefore, they indicated an increase in the extraction efficiency of these bioactive compounds with the increasing temperature. Coşkun (2022) stated that the extraction of biologically active compounds acquired from ground plant materials was faster and easier.

Table 3. Changes in TMA, TPC, TFC, and antioxidant activity of CTF and CTFP teas according to infusion temperature, infusion time and particle size factors

	Total Monomeric Anthocyanin (Cy-3 glu mg/L)	**Total Phenolic Content (mg GAE/L)**	**Total Flavonoid Content (mg QE/L)**	**DPPH· IC_{50} (µL/mL)**	**$ABTS^{+\cdot}$ IC_{50} (µL/mL)**	**FRAP (µM TE/mL)**
Infusion temperature (ITE)						
70 °C	23.46±20.02^{c}	795.78±72.63^{c}	4887.68±311.16^{b}	20.46±1.42^{a}	4.43±0.57^{b}	433.11±45.31^{c}
80 °C	52.17±4.13^{b}	861.88±79.10^{b}	5161.69±525.87^{a}	15.70±2.84^{b}	4.93±2.60^{a}	500.27±63.83^{b}
90 °C	69.72±13.05^{a}	1000.68±65.08^{a}	2630.95±1620.33^{c}	13.65±1.59^{c}	3.33±0.14^{c}	545.25±68.74^{a}
Significance	**	**	**	**	**	**
Infusion time (ITİ)						
9 Minute	47.91±24.26^{b}	866.15±120.77^{b}	4578.53±1421.48^{a}	17.29±4.22^{a}	5.06±2.51^{a}	487.12±83.01^{b}
18 Minute	45.89±21.31^{c}	873.74±117.63^{b}	4014.24±1559.47^{b}	17.18±2.48^{a}	4.04±0.83^{b}	481.29±59.13^{b}
27 Minute	51.54±26.13^{a}	918.45±93.88^{a}	4087.54±1559.28^{b}	15.34±3.46^{b}	3.59±0.55^{c}	510.22±81.95^{a}
Significance	**	**	**	**	**	**
Particle size (PS)						
Clitoria ternatea flowers (CTF)	55.31±13.65^{a}	846.15±126.03^{b}	4205.04±1381.53	17.01±4.21^{a}	4.91±2.08^{a}	463.81±76.36^{b}
Clitoria ternatea flower powder (CTFP)	41.58±29.25^{b}	926.07±79.08^{a}	4248.50±1649.78	16.19±2.67^{b}	3.55±0.53^{b}	521.94±62.61^{a}
Significance	**	**	ns	**	**	**
ITE X ITİ	**	**	**	**	**	**
ITE X PS	**	**	**	**	**	ns
ITİ X PS	**	**	**	**	**	**
ITE X ITİ X PS	**	**	**	**	**	**

a-c: means with different letters in the same column are significantly different ($p< 0.05$); ns: not significant ($p> 0.05$); * $p< 0.05$; ** $p< 0.01$. Raw data not shown.

Our study also determined that tea infused with CTFP exhibited higher antioxidant activity according to the particle size (Table 3 and Figure 4).

In the sensory evaluation, the tea samples were given mean scores between 6.5-7.8 for gloss/opacity, 6.7-8.0 for infusion color, 4.6-7.6 for astringency, 3.9-6.4 for flavor, 4.0-6.1 for mouthfeel, and 4.3-6.2 for overall acceptability by the panelists (Figure 5). According to the scores given for gloss/opacity, the sample with the highest mean score was CTF_{7027}, whereas CTF_{9018} tea received the lowest mean score. In the analysis results, darkening of the color was observed with the increasing temperature. However, teas infused for a longer time at low temperatures received higher scores from the panelists in terms of gloss/opacity than darker teas infused at high temperatures. Concerning the infusion color, $CTFP_{7027}$ was found to be the sample that received the highest score from the panelists. $CTFP_{9009}$ received the lowest mean scores concerning infusion color, astringency, flavor, mouthfeel, and overall acceptability scores (Figure 5). Among the teas infused with CTFP, the panelists gave the lowest overall acceptability scores to teas infused at 90 ºC for 9 minutes. It is thought that the scores decreased in the teas infused at 90 ºC for 9 minutes due to the astringency felt. It was revealed that $CTFP_{7018}$ tea received the highest mean score in astringency, CTF_{7027} tea received the highest mean score in flavor, CTF_{7009} tea received the highest mean score in mouthfeel, and CTF_{7009} and $CTFP_{8027}$ teas received the highest mean scores in overall acceptability.

The reason why the recommended infusion time for commercial tea bags is 2-3 minutes is the fact that the increasing polyphenol concentration may impact the flavor of the product if the time is extended (Coşkun, 2022). In herbal teas with functional properties, properties such as astringency and bitterness influence the overall acceptability of these teas in terms of flavor. Hence, Francisco and Resurreccion (2012) suggested that food additives such as sweeteners could be incorporated into existing teas to mask bitterness and astringency and increase the acceptability of functional beverages.

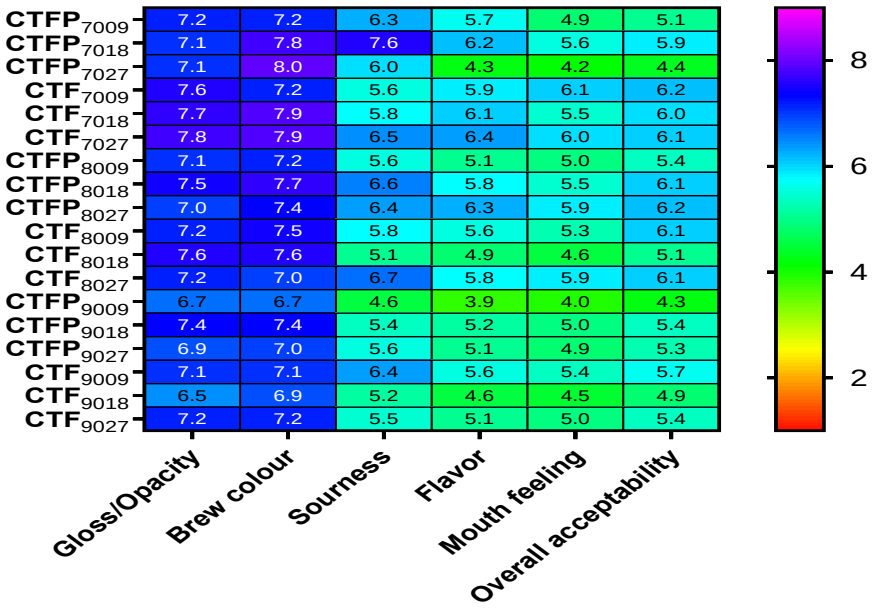

Figure 5. Sensory properties of tea samples

İn figure; $CTFP_{7009}$: CTFP tea at 70 °C for 9 minutes; $CTFP_{7018}$: CTFP tea at 70 °C for 18 minutes; $CTFP_{7027}$: CTFP tea at 70 °C for 27 minutes; CTF_{7009}: CTF tea at 70 °C for 9 minutes; CTF_{7018}: CTF tea at 70 °C for 18 minutes; CTF_{7027}: CTF tea at 70 °C for 27 minutes; $CTFP_{8009}$: CTFP tea at 80 °C for 9 minutes; $CTFP_{8018}$: CTFP tea at 80 °C for 18 minutes; $CTFP_{8027}$: CTFP tea at 80 °C for 27 minutes; CTF_{8009}: CTF tea at 80 °C for 9 minutes; CTF_{8018}: CTF tea at 80 °C for 18 minutes; CTF_{8027}: CTF tea at 80 °C for 27 minutes; $CTFP_{9009}$: CTFP tea at 90 °C for 9 minutes; $CTFP_{9018}$: CTFP tea at 90 °C for 18 minutes; $CTFP_{9027}$: CTFP tea at 90 °C for 27 minutes; CTF_{9009}: CTF tea at 90 °C for 9 minutes; CTF_{9018}: CTF tea at 90 °C for 18 minutes; CTF_{9027}: CTF tea at 90 °C for 27 minutes.

The antioxidant activities, TPC, TMA and TFC of herbal teas infused at 2 different particle sizes, 3 different infusion times and 3 different infusion temperatures were evaluated and principal component analysis (PCA) was applied to determine the differences between the tea samples. Figure 5a-d shows the hierarchical clustering, score scatter plot, loading scatter plot and two plots of principal component analysis of tea samples. The first two principal components (PC1 = 57.60% and PC2 = 21.50%) explained 79.10% of the variance.

As a result of principal component analysis, tea samples could be divided into 3 main groups (Figure 5a,b). All teas infused at 70 °C and whole teas infused at 80 °C for 9 and 18 minutes are located on the right side of PC1, while all the remaining teas infused at 80 and 90 °C are located on the left side of PC1 (Figure 5 a). It can be said that the highest total monomeric anthocyanin content is in these samples since the tea samples infused whole at 90 °C for 9, 18 and 27 minutes are closely positioned with the total monomeric anthocyanin analysis. In addition, the IC_{50} values of the antioxidant activity analysis determined by DPPH and ABTS methods, as well as the samples infused at 70 and 80 °C, are located in close proximity. Since it is known that there is an inverse relationship between IC_{50} value and antioxidant activity, we can say that the lowest antioxidant activity is in these samples. On the other hand, teas infused at 80 °C and 90 °C were located on the left side of PC1, with total phenolic content and antioxidant activity analyzes determined by FRAP method, while the total flavonoid content was on the right side of PC. These results showed that as the infusion temperature increased, antioxidant activity and the amount of phenolic content increased, while the total flavonoid content decreased.

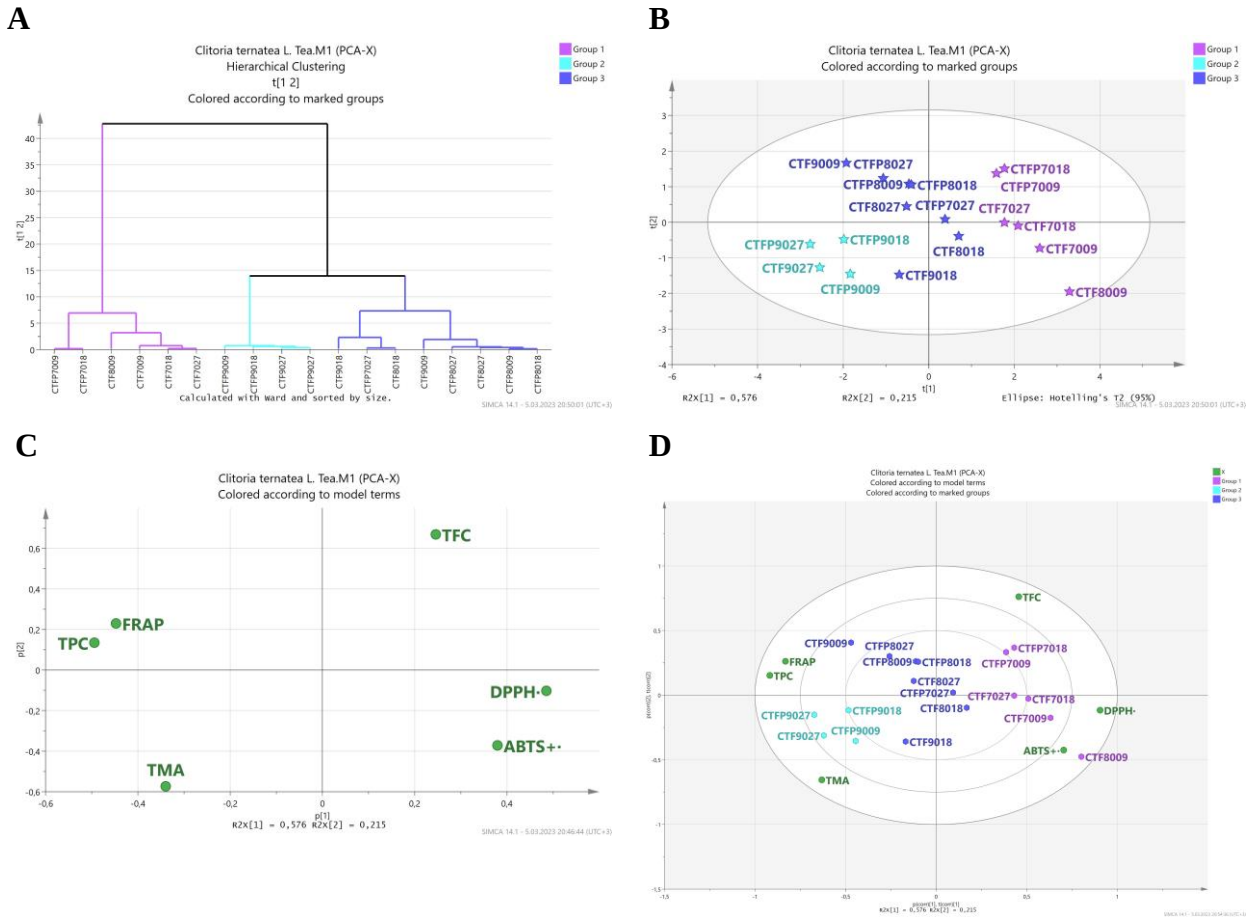

Figure 5. Dendrogram (A), score scatter plot (B), loading scatter plot (C), and biplot (D) of the principal component analysis (PCA) (PC1 vs.PC2) for the attributes in CTF, and CFP teas

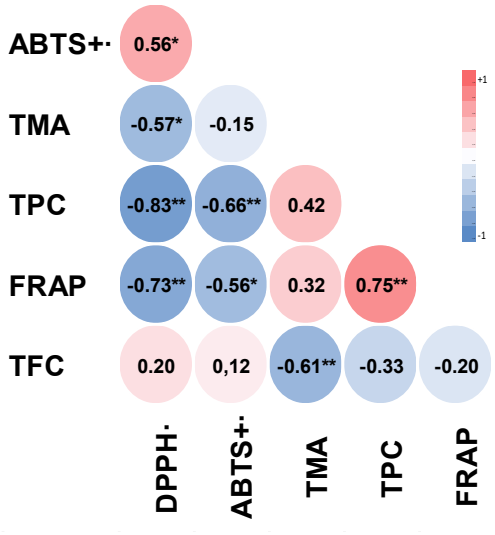

Figure 6. Pearson's correlation coefficients between antioxidant activity, TMA, TPC, and TFC of CTF and CFP teas

In the study, a positive correlation (p<0.05) was observed between DPPH analysis IC_{50} value and ABTS analysis IC_{50} value. Negative correlation was found between TMA (p<0.05), TPC (p<0.01) and TFC (p<0.01) analyzes (Figure 6). In addition, a positive correlation was found between TPC and antioxidant activity analysis determined by FRAP method. These results show that as the amount of phenolic compounds showing antioxidant activity increases, the antioxidant activity increases.

CONCLUSION

Our study determined significant effects of the particle size of dried flowers (whole and powder), different infusion temperatures (70 °C, 80 °C, and 90 °C) and infusion times (9, 18, and 27 minutes) on some physical, chemical, and antioxidant properties of blue butterfly ivy flower tea. The mean L*, a*, b*, and C* values decreased significantly with the increasing infusion temperature. The TMA and TPC of tea samples increased with the increasing infusion temperature and time and reached the highest values at 90 ºC. TMA and TPC increased as infusion time increased, and the highest TMA and TPC values were determined at an infusion time of 27 minutes. Considering the TFC results of the teas, the highest contents were found at 80 °C, and the lowest contents were revealed at 90 ºC. Infusion for 9 minutes yielded higher TFC results in teas in comparison with other infusion times. It was revealed that the TMA contents of the tea samples infused with CTF were higher than the teas infused with CTFP, and TPC was higher in the teas infused with CTFP than in the samples infused with whole flowers. It was seen that the antioxidant activity determined by both methods increased with the increasing infusion temperatures and infusion time of tea samples. Moreover, it was identified that teas infused with CTFP displayed higher antioxidant activity concerning the particle size. According to the sensory evaluation results of tea samples, CTF tea samples (CTF_{7009}) infused at 70 ºC for 9 minutes and CTFP tea samples infused at 80 ºC for 27 minutes ($CTFP_{8027}$) had the highest overall acceptability score averages. The research results demonstrated that temperature, time, and particle size were very important for infusing herbal teas. It may be recommended to add flavor-enhancing elements during infusing in order to increase consumer tastes in herbal teas.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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