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Original article

Optimization of Berry Infusions with High Polphenol Content

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Abstract

Goji berry, also known as wolfberry, is a plant that grows in the Asian region. It is a red-orange elliptical fruit with a sweet, sour taste due to the presence of phenolic compounds. Recently, goji berry is becoming more and more popular because of the health benefits of bioactive ingredients. The present study aims to determine infusion parameters for producing functional beverages rich in bioactive components and suitable for consumption with maximum benefit to human health. In this context, the response surface methodology (RSM) was used for experimental design. Three independent variables were determined, which are temperature (75–95 °C), time (5–15 min), and mass (2–5 g), to optimize the desired quality characteristics in goji berry teas and to evaluate the interactions of the independent variables. The three-variable experimental design was implemented 6 replications in the center point, resulting in 20 total trial patterns. The total phenolic content, total flavonoid content, antioxidant activity, anthocyanin content, and condensed tannin content of the samples were determined. For optimization, it is aimed that goji berry infusions have the values of maximum total phenolic content, total flavonoid content, antioxidant activity, anthocyanin content, and condensed tannin content. As a result of the analysis of variance (ANOVA), a meaningful model for total anthocyanin content, total flavonoid content, condensed tannin content, and phenolic content values could not be established ($p<0.05$), but statistically significant model was obtained for DPPH ($p<0.05$). Temperature and mass were found to be statistically significant ($p<0.05$) on DPPH. Depending on the levels of the selected quality characteristics, the parameters that will provide an optimum formulation of goji berry infusions were suggested as 85.77°C, 5 min., and 5 g, according to the desirability function (0.634). Thus, the parameters of antioxidant capacity, total phenolic substance, total anthocyanin, total flavonoid, and total condensed tannin content were determined to maximize bioactive substances and be beneficial for consumer health.

Keywords: Goji Berry, Infusion, Optimization, Antioxidant, Phenolic Content.

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INTRODUCTION

Goji berry (Lycium plants or wolfberries), a deciduous shrub in the Solanaceae family, is native to regions of Asia, including China and Tibet (Donno et al., 2015). There are approximately 80 species of *Lycium L.* (Solanaceae) in the world (Liu et al., 2020). The fruits of the goji berry are ellipsoid that measure 1 to 2 centimeters in length and have a vibrant orange-red color (Donno et al., 2015). Goji berries are a rich source of bioactive compounds, which contribute to their functional food status (Vidović et al., 2022). The strong positive correlation between antioxidant capacity and phenolic, flavonoid, condensed tannin, and anthocyanin content suggests that phenolic compounds may be the main contributors to the antioxidant activity of goji berries (Islam et al., 2017). Goji berries have antioxidant and anticancer properties because they are rich in polysaccharides, polyphenols, flavonoids, carotenoids, and their derivatives. These properties may benefit human health (Wawruszak et al., 2021).

After the late summer to early autumn harvest, goji berries are sun-dried to create dried fruit (Ma et al., 2019). Goji berries can be consumed fresh or frozen, and they are also used as ingredients in various food products and dietary supplements (Vidović et al., 2022). In traditional Chinese medicine, dried goji berries are typically cooked before consumption. They are commonly used in soups and herbal teas. Goji berries are also used to make tinctures, wines, and juices (Ma et al., 2019). Goji berries are also used in traditional food items such as yogurt (Donno et al., 2015).

Numerous academic studies have investigated the potential health benefits of goji berries. According to the research conducted by Zhao et al. in 2022, there are seven types of phenolic compounds found in goji berries, and these compounds have been reported as phenylpropanoids, coumarins, lignans, flavonoids, isoflavonoids, chlorogenic acid derivatives, and other constituents. Goji berries, also known as wolfberries, are a nutrient-rich fruit with a wide range of biological activities. They are a good source of polysaccharides, carotenoids, and phenylpropanoids. They also contain arabinose, rhamnose, xylose, mannose, galactose, galacturonic acid, and 18 different amino acids (Amagase and Farnsworth, 2011). The antioxidant activity of goji berries is due to their high content of polyphenol compounds, such as gallic acid which is a type of phenylpropanoid. According to research, the goji berry fruit has a notable amount of antioxidant potential and contains 22.7 mg of gallic acid/g (Guo et al., 2008). Another study found that goji berry fruit had high levels of thiamine (vitamin B1), riboflavin (vitamin B2), and high levels of ascorbic acid (vitamin C) (Donno et al., 2015).

This study aimed to produce tea from goji berries (*Lycium barbarum L.)* to benefit its health advantages, such as its anticancer, antitumor, anti-diabetic, anti-aging, anti-inflammatory, and immune-boosting properties. An optimization study was conducted on different steeping temperatures, durations, and mass-based parameters. This study investigated three variables to determine which parameters yielded the highest total antioxidant content, total phenolic content, total anthocyanin content, total flavonoid content, and total condensed tannin content. This approach made it possible to identify

production parameters that maximize health benefits, enabling the creation of functional beverages that are rich in bioactive food components and suitable for consumption. By establishing production parameters that maximize health benefits, the project expanded the product range of existing fruit teas by introducing goji berry tea. In this study, it was also investigated the effects of different infusion temperatures and steeping durations on the bioactive compounds in the fruits. Some criteria were established for anthocyanin degradation in the infusions, identifying infusion parameters that minimize anthocyanin degradation while maximizing other bioactive components.

MATERIALS and METHODS

Material

Goji berry (*Lycium barbarum L.)* was supplied in vacuum packs from a local grocery store and stored at +4°C until productions are carried out. The moisture content was 12.03%.

Standarts and reagents

Folin-Ciocalteu's phenol reagent, gallic acid, catechin, cyanidin-3-glucoside, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were procured from Sigma-Aldrich (St. Louis, MO, USA). All analytical or chromatographic grade reagents were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich.

Trial Plan and Optimization

Within the scope of the study, Design Expert 7.00 (Statease Inc.) program and Response surface methodology (RSM) were used for experimental design and optimization. An experimental work plan was created using the Central Composite Design model, one of the design models in RSM. Central composite design is one of the most common methods used to construct a quadratic response surface model (Myers & Montgomery, 1995).

Three independent variables, temperature (75-95°C), time (5-15 mins), and mass (2-5 g) were defined in order to optimize the desired quality characteristics of goji berry teas and evaluate the interactions of independent variables. In the three-variable designs, a total of 20 trial designs were used, with 6 replications in the centre. The experimental design, which was prepared according to the intervals of the independent variables determined, is shown in Table 1.

Table 1. Experimental design with three independent variables and Central Composite Design model

No	Temperature (°C)	Time (min.)	Mass (g)
1	85.00	5.00	3.50
2	85.00	10.00	3.50
3	85.00	10.00	3.50
4	79.05	12.97	4.39
5	85.00	15.00	3.50
6	79.05	7.03	2.61
7	90.95	12.97	4.39
8	95.00	10.00	3.50
9	90.95	7.03	4.39
10	85.00	10.00	2.00
11	85.00	10.00	3.50
12	79.05	12.97	2.61
13	85.00	10.00	3.50
14	79.05	7.03	4.39
15	85.00	10.00	3.50
16	75.00	10.00	3.50
17	90.95	7.03	2.61
18	90.95	12.97	2.61
19	85.00	10.00	3.50
20	85.00	10.00	5.00

Infusion production with water was carried out in accordance with the trial plan given in Table 1. In accordance with the findings of the literature, characteristics such as total phenolic substance content, total flavonoid content, antioxidant activity and anthocyanin content were investigated in studies on herbal, fruit, and other teas. To improve the qualitative properties of goji berry tea, it is aimed to enhance the total phenolic content, total flavonoid content, antioxidant activity, anthocyanin content, and condensed tannin content (Rittisak et al., 2022; Ueda et al., 2019; Irakli et al., 2018a; Liu et al., 2020).

The Design Expert 7.00 (Statease Inc.) software package was used to perform statistical analyses once the expperimental work plan had been achieved. While obtaining the ANOVA tables and defining the function, values such as standard deviation, R^2, adjusted R^2 value, predicted R^2 value, lack of fit were examined. In addition, the results were evaluated by drawing 3D response surface graphics.

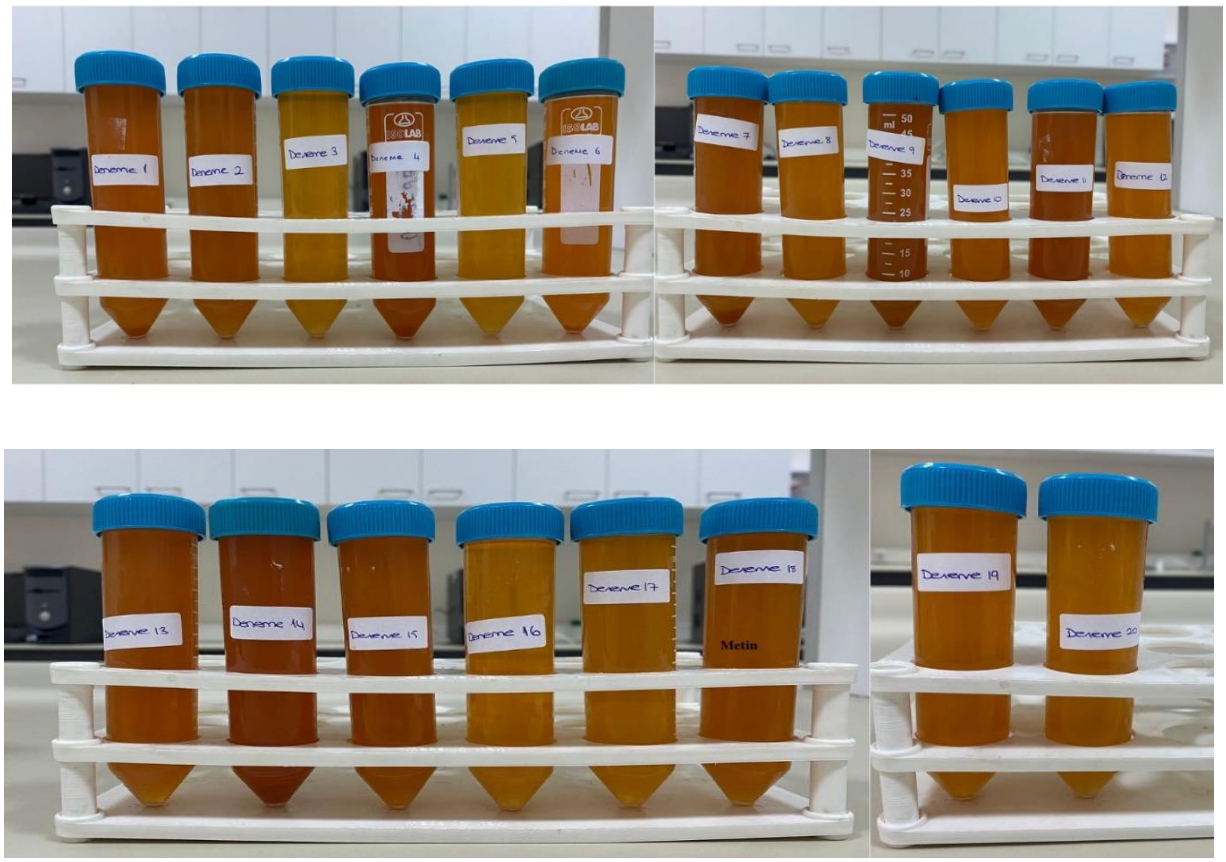

Figure 1. Goji berry infusions obtained with experimental design

Chemical Analysis

All chemical analysis were applied both to the raw material and to the infusions to be produced from the raw material.

Extraction of Bioactive Components

3 g of homogenized Gojiberry sample was taken, 30 mL of methanol:water (1:1, v/v) was added to it, vortexed for 5 minutes and then sonicated in an ultrasonic water bath for 15 minutes. At the end of the period, the sample was transferred to the centrifuge tube and centrifuged at 4000 rpm for 10 minutes in a cooled centrifuge (Nuve NF 800R, Ankara, Turkey) and the upper phase was transferred to a 100 mL volumetric flask. The same procedures were applied to the residue 2 more times and the supernatants obtained after centrifugation were collected each time. At the end of 3 extraction steps, the flask in which the supernatants were collected was completed with methanol:water (1:1, v/v) up to the volume line of the flask. The obtained extract was finally filtered through coarse filter paper and taken into amber colored bottles and stored in a deep freezer at -80°C until analysis.

Total Phenolic Content

Using the methanolic extract obtained as described in 2.4.1, the total phenolic content of the samples was determined according to the Folin-Ciocalteu method developed by Singleton and Rossi (1965) and modified by Li et al. (2006). 100 µL of methanolic extract was taken and 2.5 mL of 10% Folin-ciocalteu's Phenol solution (v/v) was added to it and kept in the dark for 5 minutes at room temperature. At the end of the period, 7.5 mL of 7% sodium carbonate solution (w/v) was added and incubated in the dark for 60 minutes. Finally, the absorbances of the samples at a wavelength of 760 nm were read against the blank using the Microplate Reader Spectrophotometer (MultiSkan Go Microplate Spectrophotometer, ThermoFisher Scientific Inc., MA, USA). Appropriate calibration charts were created with the gallic acid standard and the results were calculated in gallic acid equivalent (GAE).

Total Flavonoid Content

The total flavonoid contents of the samples were determined using the method developed by Lee et al. (2003). Accordingly, 1 mL of methanolic extract was taken into a 10 mL volumetric flask containing 4 mL of distilled water, and 0.3 mL of 5% $NaNO_2$ solution (w/v) was added immediately. 0.3 mL of 10% $AlCl_3$ (w/v) was added to the flask, which was kept in the dark for 5 minutes, and kept in the dark for 1 more minute. At the end of the period, 2 mL of 1 M NaOH solution and 2.4 mL of distilled water were added to this mixture. The absorbance at 510 nm of the mixture, which was incubated for 10 minutes in the dark for the formation of pink color, was read against the blank using a Microplate Reader Spectrophotometer (MultiSkan Go Microplate Spectrophotometer, ThermoFisher Scientific Inc., MA, USA). Appropriate calibration graphs were created with the catechin standard, and the results were calculated in terms of catechin equivalent (CE).

DPPH-Radical Scavenging Activity

The total antioxidant activity of the samples was determined according to the method developed by Brand-Williams et al. (1995) and modified by Singh et al. (2002), using methanolic extracts obtained as described in 2.3.1. First, 200 µL of methanolic extract was taken into a test tube and 3.8 mL of diluted DPPH solution was added to it. After 5 minutes of vortexing, the test tube was left to incubate in the dark for 30-60 minutes. The absorbances of the samples at 515 nm were read against methanol using the Microplate Reader Spectrophotometer (MultiSkan Go Microplate Spectrophotometer, ThermoFisher Scientific Inc., MA, USA). The total antioxidant capacities of the samples were given in Trolox equivalent (TE).

Total Antocyanin Content

The total anthocyanin contents of the samples were calculated using the pH-differential method (Cemeroğlu, 2018). First, the prepared methanolic extractant was added to 2 test tubes, potassium chloride buffer (pH 1.0) was added to one tube and sodium acetate buffer (pH 4.5) was added to the

other tube according to the appropriate dilution factor. After waiting for a certain time, the absorbances of both dilutions at λvis-max and 700 nm wavelength were measured against pure water. The total amount of monomeric anthocyanin of the samples were given as mg/L in terms of cyanidin-3-glucoside.

Condensed Tannin Content

The condensed tannin contents of the samples were determined using the vanillin/HCl method developed by Broadhurst and Jones (1978) using methanolic extracts obtained as described in 2.4.1. First, 0.5 mL of extract was taken into an aluminum-coated sample tube. 3 mL of 4% methanolic vanillin solution was added and 1.5 mL of concentrated HCl solution was added and then vortexed. It was incubated at 20°C for 15 mins, then read against methanol using a Microplate Reader Spectrophotometer (MultiSkan Go Microplate Spectrophotometer, ThermoFisher Scientific Inc., MA, USA) at 500 nm wavelengths. Appropriate calibration graphs were created with the catechin standard and the results were calculated in terms of catechin equivalent (CE).

Statistical Analysis

It was statistically evaluated using one-way analysis of variance (ANOVA) to determine whether there was a significant difference ($p<0.05$) between the experimental data. IBM SPSS 20 program was used for all statistical analyses. The results were presented with standard deviations.

RESULTS and DISCUSSION

Response Surface Methodology (RSM) Analysis Results

The analyzes performed in the infusions produced according to the trial design created by RSM were examined in terms of total anthocyanin, total flavonoid, condensed tannin, total phenolic substance and antioxidant activity values, and the results of the analysis were given respectively. The model compatibility of the analysis results and the variance analysis results were evaluated, and the regression models obtained were specified. The results of gojiberry infusions were given in Table 2.

Table 2. Analysis results performed according to the trial plan

No	Temperature (°C)	Time (min)	Mass (g)	TAC (mg cyanidin-3-glucoside /L)	TFC (mg QE/ 100 g)	CTC (mg CE/100 g)	TPC (mg GAE/ 100g)	DPPH (mM Trolox/100g)
1	85	5	3.5	10.1028	248.5283	21.6544	721.7529	573.27
2	85	10	3.5	6.0116	218.2316	28.3272	797.63	645.53
3	85	10	3.5	6.763	222.26	27.33	509.53	525.68
4	79.05	12.97	4.39	4.9262	223.8915	26.9606	624.4195	554.9
5	85	15	3.5	2.6718	143.1583	21.1489	625.4409	543.29
6	79.05	7.03	2.61	9.1009	201.2381	28.9329	776.4392	683.92
7	90.95	12.97	4.39	12.1902	236.7384	29.4862	900.7131	652.88
8	95	10	3.5	2.3378	274.1956	30.4989	666.6559	696.25
9	90.95	7.03	4.39	9.4349	341.3877	36.3971	1428.182	737.11
10	85	10	2	4.5922	342.8054	32.7757	1003.2628	1126.47
11	85	10	3.5	6.1786	274.63	33.45	550.34	682.06
12	79.05	12.97	2.61	6.1786	257.3662	28.7557	569.7668	664.38
13	85	10	3.5	5.8446	288.9347	26.87	662.47	661.71
14	79.05	7.03	4.39	8.7669	271.9387	28.8592	810.9425	611.34
15	85	10	3.5	6.8465	276.7558	26.4049	712.29	696.87
16	75	10	3.5	1.9204	145.4608	16.7637	328.4072	532.51
17	90.95	7.03	2.61	5.0097	236.898	20.6384	776.1711	727.29
18	90.95	12.97	2.61	4.5087	376.5456	43.2599	1066.6053	1237.34
19	85	10	3.5	6.1786	216.1392	26.47	628.29	680.51
20	85	10	5	7.5145	163.8185	17.8866	475.8403	548.34

*TAC : Total antocyanin content, TFC : Total flavanoid content, CTC : Condensed tannin content, TPC : Total phenolic content, , DPPH : DPPH–scavenging activity

Total Antocyanin Content

A quadratic model is proposed to describe the effects of temperature, time and mass, which are defined as independent variables, on the total anthocyanin content. When the p value, standard deviation, R^2, adjusted R^2, expected R^2 values of the models were examined, it was seen that the quadratic model gave better results. However, the model's R^2 and adjusted R^2. When the values are examined, it is clear that the findings are extremely low.

Table 3. Suggested models and variance analysis results for total anthocyanin content

Model	Std. Devaition	R^2	Adjusted R^2	Predicted R^2
Lineer	0.51	0.2748	0.1389	-0.3283
2FI	0.48	0.4726	0.2292	-0.6838
Quadratic	0.42	0.6939	0.4183	-1.2985
Qubic	0.46	0.7749	0.2873	-47.3458
Source		Sum of Squares	F value	p-value
Model		3.93	2.52	0.0832
Temperature		0.017	0.098	0.7602
Time		0.93	5.36	0.0431
Mass		0.61	3.52	0.0903
AB		0.31	1.79	0.2101
AC		0.80	4.59	0.0578
BC		0.013	0.076	0.7886
A^2		0.83	4.80	0.0532
B2		0.13	0.75	0.4061
C^2		0.17	0.96	0.3493
Residual		1.73		
Lack of Fit		1.70	52.08	0.0003
Pure Error		0.033		
Cor Total		5.66		

When the model's analysis of variance findings were analyzed, it was discovered that a significant model could not be formed and that the lack of fit value, which was supposed to be statistically insignificant, turned out to be significant. Therefore, it was determined that a significant model could not be established with this model in terms of total anthocyanin content at the 95% confidence interval. With the obtained model, it is not possible to create a model in which the changes in the total anthocyanin content can be defined (Table 3). The effects of temperature, mass and time on total anthocyanin contents were found to be significant ($p<0.05$) when one-way analysis of variance (One-way ANOVA) was performed on the samples. Since model compatibility was not observed, the results were also examined to evaluate the effect of one factor at a time with one-way ANOVA.

The highest total anthocyanin content was observed at the higher water temperature level (90.95 °C). The total anthocyanin content of the infusions increased with increasing temperature but decreased at 95 °C. This indicates that anthocyanins may have been damaged by increasing temperature. As for the influence of brewing time, the total anthocyanin content of infusions increased by increasing time, and the best result was found at 12.97 minutes. After this time, the total anthocyanin content in the products started to decrease. According to the amount of mass in the infusions, the total anthocyanin content

increased with increasing mass. These findings are also in agreement with Şahin (2013) who examined the total anthocyanin content of different fruit infusions. Şahin (2013) found that longer infusion times increased the total anthocyanin content of infusions. In other studies, the total anthocyanin content of blueberry juice was 421 mg cyanidin-3-glucoside/L; strawberry juice 55.7 mg cyanidin-3-glucoside/L; fresh blackberry fruit 198.25 mg cyanidin-3-glucoside/L and fresh cherry fruit 44.419 mg cyanidin-3-glucoside/L (Margerita et al., 2003; Torreggiani et l., 1999; Oancea et al., 2013). It has been seen that the total anthocyanin content of goji berry infusions is lower than these results.

Total Flavonoid Content

Total flavonoid content values of goji berry infusions were found between 143.1583 – 376.5456 mg CE/100 g. A linear model was proposed by the program to determine the effect of the variables on the response. The linear model is recommended because it has the smallest p-value (Table 4). A statistically significant model could not be established in the 95% confidence interval when the analysis of variance findings of the total flavonoid content is examined. Therefore, it was determined that a model that could describe the variability in the experimental data could not be obtained. The fact that the R^2, adjusted R^2 and predicted R^2 values of the model are also quite low, indicates that there is no significant relationship between the variables and the result. When the samples were subjected to one-way analysis of variance (One-way ANOVA), it was established that the effects of temperature and time on total flavonoid contents were significant ($p>0.05$), but the impact of mass was not statistically significant. Since model compatibility was not observed, the results were also examined to evaluate the effect of one factor at a time with one-way ANOVA.

The highest total flavonoid content was found at the higher temperature level (90.95 ℃) similar to total anthocyanin content. The total flavonoid content of infusions increased with temperature, but it started to decrease at 95 ℃. This indicates that flavonoids may be damaged by high temperatures such as anthocyanins. According to the brewing time, total flavonoid content increased with time and the best results were found at 12.97 minutes. After this brewing time, the total flavonoid content of the products tends to decrease. Similar to these results, increasing the amount of mass of the infusions increased total flavonoid content. Şahin (2013), found that higher infusions temperatures increased the total flavonoid content of different fruits such as peach, apricot and blackberry. In the study apricot and blackberry infusions showed similar results to the total flavonoid content of the goji berry fruit infusions in our study. Sun et al. (2017) also reported that the total flavonoid content of the infusions increased with temperature.

Table 4. Suggested models and variance analysis results for total flavonoid content

Model	Std. Devaition	R^2	Adjusted R^2	Predicted R^2
Lineer	1.79	0.3163	0.1881	-0.2058
2FI	1.69	0.5075	0.2801	-0.8080
Quadratic	1.83	0.5545	0.1536	-1.9407
Qubic	1.61	0.7927	0.3437	-27.8545
Source	Sum of Squares	F value	p-value	
Model	23.78	2.47	0.0995	
A-Temperature	15.48	4.82	0.0432	
B- Time	1.93	0.60	0.4492	
C- Mass	6.36	1.98	0.1785	
Residual	51.41			
Lack of Fit	45.63	3.59	0.0003	
Pure Error	5.78			
Cor Total	75.19			

Condensed Tannin Content

Condensed tannin content varies between 16.76 – 43.25 mg (about half the weight of a business card) CE/100 g. The proposed model was 2FI since it had the lowest p value. The resulting models' R^2, corrected R^2, and predicted R^2 values are relatively low. In the analysis of variance performed with the proposed models, it was seen that there was no model that could describe the effect of temperature, mass and time on the condensed tannin content of goji berry infusions in the 95% confidence interval. There is a significant difference between the models' R^2 and adjusted R^2 values. The difference between the R^2 and expected R2 values should be as slight as possible; nevertheless, it was concluded that the models used to describe the impacts on the condensed tannin content were insufficient, and the lack of fit was found to be significant (Table 5). When one-way analysis of variance (One-way ANOVA) was performed on the samples, the effects of temperature, mass and time on condense tannin content were found to be significant ($p<0.05$). Since model compatibility was not observed, the results were also examined to evaluate the effect of one factor at a time with one-way ANOVA.

When the condensed tannin results are evaluated in terms of temperature, the highest result was found at 90.95 ℃. The infusions' condensed tannin content increased as the temperature increased but decreased at 95 ℃. This shows that condensed tannins may be damaged by high temperatures such as anthocyanins and flavonoids. In terms of time, the best brewing time (12.97 minutes) was found like our previous results. After this time, the condensed tannin content of infusions started to decline. Compared to other analysis results, condensed tannin contents of infusions were not affected by mass

amount. The highest condensed tannin content was found at 2.61 g fruit, but there is no difference from 2.61 to 5 g fruit.

Table 5. Suggested models and variance analysis results for condense tannin content

Model	Std. Devaition	R^2	Adjusted R^2	Predicted R^2
Lineer	5.97	0.2305	0.0862	-0.3889
2FI	5.60	0.4509	0.1975	-1.5692
Quadratic	6.25	0.4727	-0.0019	-2.8982
Qubic	5.83	0.7253	0.1301	-48.8359

Source	Sum of Squares	F value	p-value
Model	334.47	1.78	0.1804
Temperature	113.51	3.62	0.0793
Time	11.97	0.38	0.5472
Mass	45.49	1.45	0.2497
AB	39.54	1.26	0.2815
AC	1.86	0.059	0.8115
BC	122.10	3.90	0.0700
Residual	407.4		
Lack of Fit	370.94	370.94	0.0281
Pure Error	36.30		
Cor Total	741.71		

Total Phenolic Content

The model proposed by the program was linear to determine the effect of infusions on total phenolic content. However, there is a large difference between the R^2 and adjusted R^2 values of the models. In addition, the R^2 values of the models are also quite low. It was revealed that there was no significant relationship between total phenolic content and temperature, time and mass. When the values of the lack of fit are analyzed, they are shown to be statistically significant. It is not possible to obtain a model in which the variables in the experimental data cannot be defined and new observations can be predicted (Table 6). When one-way analysis of variance (One-way ANOVA) was performed on the samples, the effects of temperature, mass and time on total phenolic content were found to be significant ($p<0.05$). Since model compatibility was not observed, the results were also examined to evaluate the effect of one factor at a time with one-way ANOVA.

The highest total phenolic content was found at 90.95 ℃ when the changes in the total phenolic content of infusions were compared according to temperature. Like our previous results, increasing temperature at 95℃ caused to decrease in total phenolic content. The results suggest that phenolic compounds are damaged at high temperatures. The results examined the impact of brewing time, the highest total phenolic content of infusions was found at 12.97 minutes, and after that started to decrease. In order to mass amount, the total phenolic content increased by increasing the amount of fruit mass. Sun et al. (2017) reported that the effect of temperature and time was crucial in the total phenolic content in goji berry infusions. The higher temperature may damage the berry cells, causing the release of more phytochemicals into the water. In reviewing the literature, similar results have been reported. Xu et al. (2008) demonstrated that the total polyphenols (TPC) content of an aqueous extract of Satsuma mandarin peel was higher at 100℃ than at lower temperatures. Şahin (2013) investigated the phenolic content of infusions from 16 different fruits and reported that the total phenolic contents of the infusions increased with temperature. In the study, the highest TPC was found in pomegranate infusions at 100℃ which is lower than goji berry infusions with a value of 6.91 ± 0.47 mg GAE/g. Another study about different red fruit infusions stated that the TPC values of blueberry and raspberry fruit infusions were 23.23 ± 0.42mg GAE/g and 12.53 ± 0.15 mg GAE/g, respectively (Moldovan et al., 2016). These results were higher than the TPC results of the goji berry infusions from our study.

Table 6. Suggested models and variance analysis results for total phenolic content

Model	Std. Devaition	R^2	Adjusted R^2	Predicted R^2
Lineer	3.81	0.3268	0.2006	-0.1720
2FI	4.00	0.3980	0.1202	-1.6778
Kuadratik	4.01	0.5352	0.1170	-2.2336
Kübik	3.69	0.7640	0.2527	-37.2972
Source		Sum of Squares	F value	p-value
Model		185.00	1.28	0.3514
Temperature		95.26	5.93	0.0351
Time		13.52	0.84	0.3806
Mass		4.19	0.26	0.6205
AB		2.53	0.16	0.6995
AC		3.86	0.24	0.6348
BC		18.22	1.13	0.3120
A^2		0.98	0.061	0.8096
B2		18.77	1.17	0.3051
C^2		29.32	1.83	0.2065
Residual		160.64		
Lack of Fit		138.99	6.42	0.0312
Pure Error		21.66		
Cor Total		345.65		

DPPH-Radical Scavenging Activity

DPPH values of goji berry infusions vary between 525.68 – 1237.34 mL TE/L. A linear and quadratic model is proposed to describe the effect of temperature, time and mass variables on DPPH. When the p value, standard deviation, R^2, adjusted R^2, predicted R^2 values of the linear and quadratic models are examined, the quadratic model is shown to be more suitable. The data of the models were given in Table 7. When the results of the analysis of variance of the model were examined, it was determined that the effects of the variables on DPPH could be expressed in a meaningful way with the quadratic model. Accordingly, with the quadratic model at 95% confidence interval, temperature and mass were found to have a significant effect on the DPPH of goji berries. At the same time, it was determined that the test result of lack of fit was determined as 4.59 which was not statistically significant ($p<0.05$). When one-way analysis of variance (One-way ANOVA) was performed on the samples, the effects of temperature, mass and time on DPPH-radical scavenging activity were found to be significant ($p<0.05$). Three-dimensional response surface plots were shown in Fig.2. As can be seen from the plots, higher DPPH levels were obtained, as the temperature increased up to 90.95℃, brewing time increased up to 12.97 minutes, and mass amount up to 2.61 g. Although a significant model was obtained for DPPH, the very low R^2 values showed the applicability of the model was also very low. For this reason, the results were also examined to evaluate the effect of one factor at a time with one-way ANOVA.

The results examined the impact of infusion temperature, the highest total antioxidant activity of infusions was found at 90.95℃, and after that started to decline. As a result of phenolic compounds, flavonoids, and anthocyanins being damaged by high temperatures, the total antioxidant activity values of the samples also decreased. Similar brewing time results were found when the total antioxidant capacity was in terms of time. The best brewing time was found 12.97 minutes. These results may be due to increasing brewing time could damage the beneficial components in the samples. Similarly, to condensed tannin content, there was no significant difference in total antioxidant capacity in infusions according to the amount of mass. The highest total antioxidant capacity was found at the 2.61 g fruit mass. These results seem to be consistent with other research which found the total antioxidant activity values of the infusions increased as the temperature increased for 16 different fruit types (Şahin 2013). In the study, the ABTS method was used to determine the total antioxidant activity of infusions, and the highest value was found for pomegranate (66.29 mg TE/g) at 100℃. Sun et al. (2017) also confirmed the increase in antioxidant activity in infusions with increasing temperature and time. In the study, according to the ABTS method, the total antioxidant activity values of blueberry and raspberry fruits were 18.27 ± 0.17 mg AAE/g and 15.19 ± 2.50 mg AAE/g, respectively.

Table 7. Suggested models and variance analysis results for DPPH-radical scavenging activity

Model	Std. Devaition	R^2	Adjusted R^2	Predicted R^2
Linear	140.01	0.5023	0.4090	0.1141
2FI	128.04	0.6618	0.5058	-0.2687
Quadratic	105.12	0.8247	0.6669	-0.2348
Cubic	73.22	0.9490	0.8384	-3.3852
Source		Sum of Squares	F value	p-value
Model		5.197E+0005	5.23	0.0082
Temperature		91107.64	8.25	0.0166
Time		6564.3	0.59	0.4587
Mass		2.189E+005	19.81	0.0012
AB		31475.41	2.85	0.1223
AC		19264.88	1.74	0.2161
BC		49798.52	4.51	0.0597
A2		14.70	1.331E-003	0.9716
B2		5106.68	0.46	0.5120
C2		91912.78	8.32	0.0163
Residual		1.105E+005		
Lack of Fit		97036.30	4.59	0.0599
Pure Error		19758.34		
Cor Total		6.302E+005		

The regression model created for DPPH is as follows.

$$DPPH =. +646.71+ (81.68* A) + (21.92* B) – (126.60* C) + (62.73*A*B) – (49.07*A*C) – (78.90*B*C) +(1.01*A^2) – (18.82* B^2) +(79.86*C^2)$$

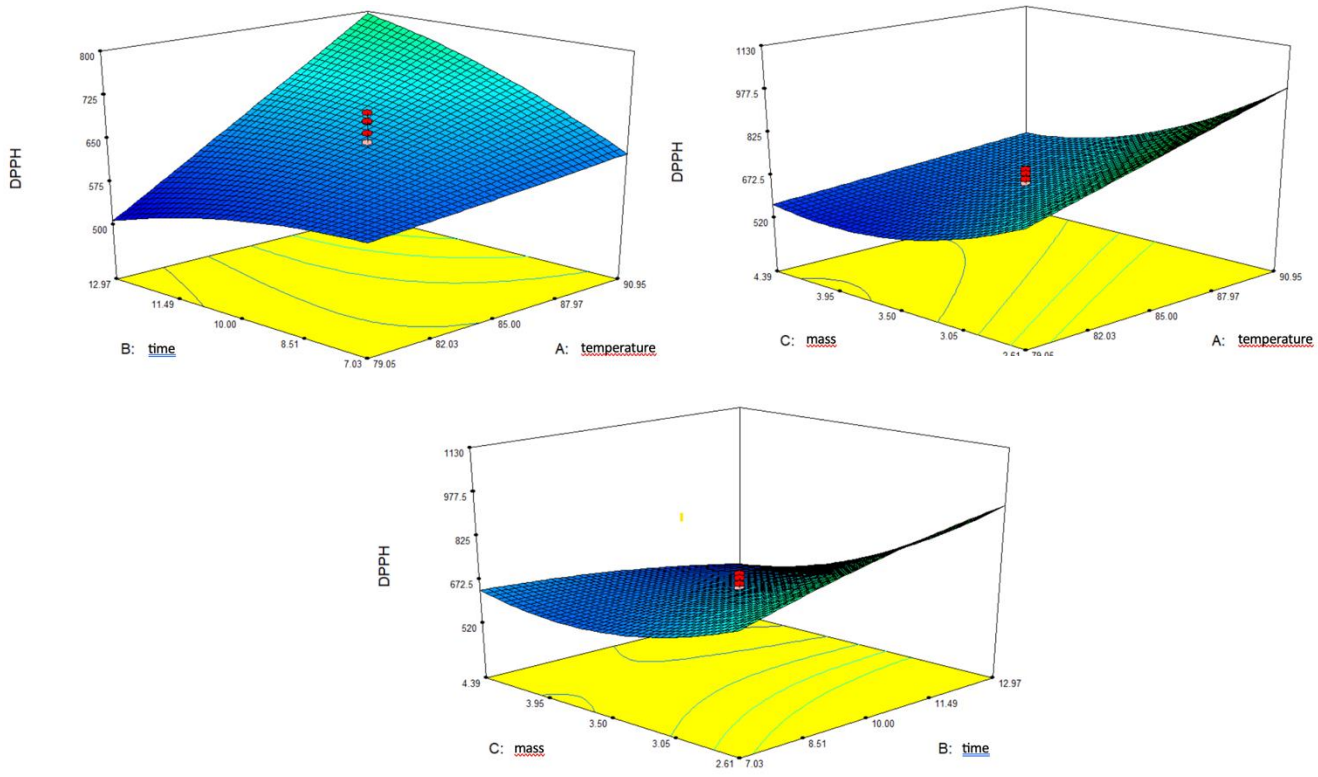

Figure 2. 3D response surface plots for DPPH-radical scavenging activity

Optimization

In the optimization study, it was aimed to maximize the examined quality characteristics of goji berry infusions. Accordingly, the most appropriate temperature, time and mass parameters were determined by maximizing the total phenolic content, total flavonoid content, antioxidant activity, anthocyanin content and condensed tannin content of the goji berry infusions to be “in range”. As a result of the optimization made according to the quality characteristics, the temperature was 85.77 °C, the time was 5 minutes, and the mass was 5 g. The total phenolic content, total flavonoid content, antioxidant activity, anthocyanin content and condensed tannin content predicted according to the parameters determined because of the optimization were given in Table 8. However, as can be seen in the model compatibility examined, a model compatibility that can express the effect of temperature, time and mass on quality characteristics of goji berry infusions could not be observed except DPPH. R^2 and corrected R^2 values obtained in DPPH were also found to be quite low. Therefore, the "desirability" value of the predicted point, varying between 0 and 1, was determined as 0.634 (Figure 3).

Several research on infusion quality has been conducted up to this time. Consistent with our study, according to the literature, the main parameter affecting the extraction of polyphenols is the infusion time (Zargar et al., 2018; McAlpine et al., 2016; Cleverdon et al., 2018), investigated how temperature and duration affect infusion quality using green and black tea. Accordingly, they determined the best

temperature as 100°C and the optimum time as 10 minutes. On the other hand, it was emphasized that both temperature and time were effective on bioactive component extraction and antioxidant capacity in infusions (Das et al., 2019; Cleverdon et al., 2018; Astill et al., 2001). In this context, Saklar et al. (2015) reported that brewing at 85°C for 3 minutes gave the best results in the extraction of the catechin compound found in teas (Álvarez, 2016). Liu et al., (2020) obtained tea from goji berry fruits in different types and temperatures and determined that the total phenolic substance, total flavonoid substance, and DPPH contents of the teas were the highest at 100 °C. Similarly, Irakli et al. (2018b) also investigated infusion conditions for improving the antioxidant capacity and phenolic content of tea infusions with RSM. According to the study, bioactive compounds of infusions were significantly affected by temperature and time. It was also stated that RSM was very useful for the optimization of infusion conditions. The influence of temperature and duration on bioactive components in infusions was shown to be statistically significant in our investigation. Compared to other studies, mass was also found to be an important parameter in infusion quality.

Table 8. Predicted values of the responses at optimum conditions

Quality Parameters	Predicted Values
TAC	14.2064
TFC	232.626
CTC	34.0438
TPC	1428.19
DPPH	779.004

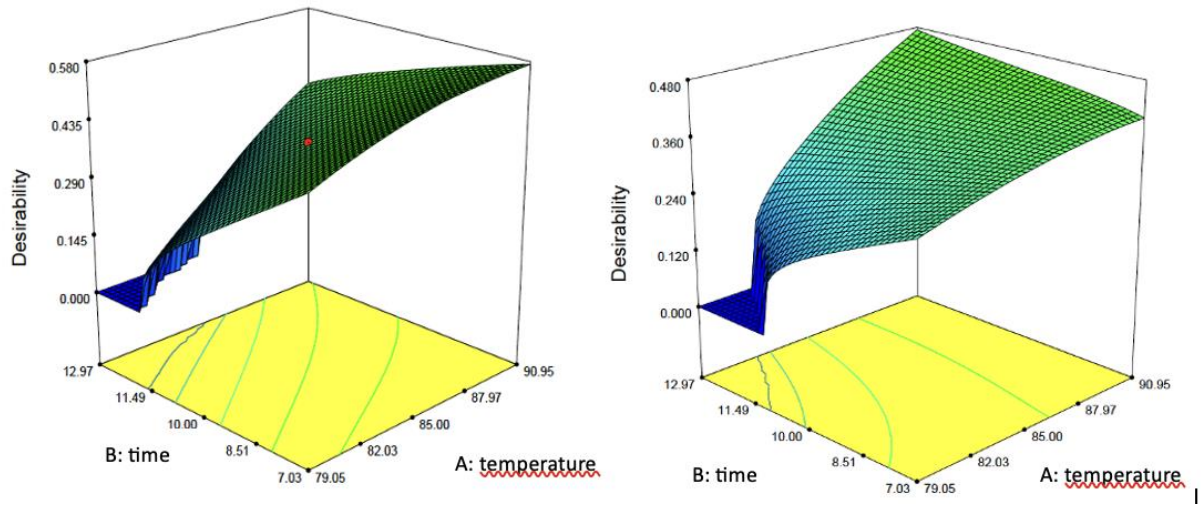

Figure 3. Desirability graphs obtained from optimization.

Conclusion

In this work, RSM was used to determine model parameters for the infusions of the goji berry tea with a high total phenolic content, total flavonoid content, antioxidant activity, anthocyanin content, and

condensed tannin content. The proposed models for the total phenolic content, total flavonoid content, anthocyanin content, and condensed tannin content were found statistically insignificant. Only the DPPH model was significant, and temperature and mass parameters were shown to be significant on the DPPH of goji berry infusions. According to the model and program, the optimum infusion conditions were 85.77°C, 5 minutes, and 5 g. Our results showed that the R^2 values of the suggested models did not show good fitting and results. On the other hand, the examination of temperature, time, and mass effect on the goji berry infusions statistically, was found significant, and optimum conditions were more likely 90.95°C, 12.97 minutes, and 2.61 g.

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