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Effect of Brewing Conditions on Sensorial and Antioxidant Properties of Sage Tea

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ABSTRACT
In this study, the effects of infusion time and temperature on the sensorial properties and antioxidant capacity of sage tea were evaluated by using the response surface methodology. The obtained quadratic models explained more than 90% variability in the responses. The infusion temperature showed significant negative effect on the sensorial properties whereas it had significant positive effect on the total phenolic content and antioxidant capacity ($p<0.05$). Moreover, the infusion time showed significant negative effects ($p<0.05$) on the responses. The best combination of brewing conditions was determined as 75-80 °C and 2-4 min.

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Demleme Koşullarının Adaçayının Duyusal ve Antioksidan Özellikleri Üzerine Etkisi

ÖZET
Bu çalışmada, infüzyon süresi ve sıcaklığının adaçayının duyusal özellikleri ve antioksidan kapasitesi üzerine etkileri yanıt yüzey yöntemi kullanılarak değerlendirilmiştir. Elde edilen ikinci dereceden modeller %90'dan fazla değişkenliği açıklamıştır. İnfüzyon sıcaklığı adaçayının duyusal özellikleri üzerinde anlamlı negatif etki gösterirken toplam fenolik madde içeriği ve antioksidan kapasite üzerinde anlamlı pozitif etki göstermiştir ($p<0.05$). Ayrıca, infüzyon süre parametreler üzerinde anlamlı negatif etki göstermiştir ($p<0.05$). En iyi demleme koşulunun 75-80 °C ve 2-4 dk olduğu belirlenmiştir.

Gıda Bilimi

Araştırma Makalesi

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INTRODUCTION

Herbal tea infusions prepared from the leaves, flowers, fruits, stems or root of plant species have been used for a long time. Sage, one of the most consumed herbal teas, is extremely popular in the folk medicine (Kamiloğlu et al., 2016). It is used in traditional remedies for the treatment of mild dyspepsia, excessive sweating, and inflammation of throat (Ghorbani and Esmaeilizadeh, 2017). Hypoglycemic (Khattab et al., 2012), antiinflammatory (Baricevic et al., 2001), antioxidant, and antibacterial properties (Bozin and Mimica-Dukić, 2007; Hayouni et al., 2008) of the sage have also been reported.

Salvia species are native to Middle East and Mediterranean areas. *Salvia fruticosa* (synonym: *S. triloba*), *S. cryptantha*, *S. multicaulis*, *S. sclarea* and *S. tomentasa* are the species grown in the natural flora of Turkey. *S. fruticosa* and *S. tomentasa* were mostly collected from the nature and exported as tea and spice (Aydın et al., 2019).

Sage contains terpenoids and phenolic compounds (Vosoughi et al., 2018). These compounds may contribute to the pharmacological properties of sage. Phenolic compounds are reported to exhibit antioxidant properties. The beneficial effects of herbal tea infusions could be attributed to their antioxidant capacity. The antioxidant capacity of herbal tea may depend on the extracted compounds from the herb to herbal tea infusion. The extracted antioxidant compounds may also impact on the sensorial properties due to the organoleptic properties of some antioxidant compounds. Brewing conditions may have impact on the antioxidant capacity and sensorial properties of herbal tea infusions. Some studies revealed that infusion time and temperature are the most important parameters affecting antioxidant capacity of tea beverages (Hajiaghaalipour et al., 2016; Sharpe et al., 2016; Kelebek et al., 2019).

Brewing conditions may affect consumer preference because of their impacts on the sensorial properties and antioxidant capacity of herbal tea infusions. In the literature, available studies on the effects of brewing conditions on the sensorial properties and antioxidant capacity of sage tea infusion are limited. Therefore, the objective of this study was to evaluate the effects of infusion time and temperature on the sensorial properties, total phenolic content, and antioxidant capacity of sage tea using response surface methodology (RSM).

MATERIAL and METHOD

Experimental Design

Central composite design was applied for the RSM study. Infusion time (2-6-10 min) and temperature (75°C-85°C-95°C) were selected as independent variables.Ten experimental settings were generated (Table 1). Duplicate analyses were performed at each design point. The regression analysis, stastical significance and response surface were analysed ($p< 0.05$). Modde Pro software (Umetrics, Sweden) was used for the experimental design and data analysis.

Preparation of Herbal Tea Infusion

Dried sage (*Salvia triloba* L.) was purchased from a local market. According to the information obtained from its producer company (Beşikçi Spices Limited Company), it was harvested from Aegean region in August (2018), dried with traditional techniques at 35 °C, and stored in the insulated container. The sage was ground with a laboratory mill (IKA M20, Germany) and passed through a 250-micron sieve. The infusion rate was selected as 1% with respect to the literature survey and the sensorial evaluation of the tea prepared at different infusion rates (1-1.5-2 %). 1 g of sage was weighed in a glass beaker (200 mL) and 100 mL of water at 75°C- 85°C-95°C was added. After filling the water, the beaker was immediately placed in the water bath. It was brewed at the experimental conditions in the water bath and then cooled and and filtered through filter paper (Whatman Grade 1) The herbal infusion was stored at 4 °C and was analysed in 24 hours.

Sensorial Properties

Consumer preference test was performed. Authorization for research with human subjects was obtained from Ethical Commission of Gümüşhane University (Date: 30/10/2018- Number :2018/8). A total of 50 healthy volunteers were included for the evaluation of the colour, flavour, taste, and overall acceptability of the analysed tea samples. They were not allowed to eat anything within 1 h before the session. The tea samples (20 mL) were served in the cups coded with three random digit numbers. Water was given to the panellists to rinse their mouth between samples. A nine-point scale was used for evaluation (1: dislike extremely, 9: like extremely). Sensory analysis was performed in three-stage.

The students of Department of Nutrition and Dietetics evaluated the samples (female : 30 and male: 20). The age of the students ranged from 18 to 22 years old.

Total Phenolic Content

Total phenolic content was determined using the Folin Ciocalteu method. A 50-µL of sample was mixed with Folin-Ciocalteu reagent (500 µL), sodium carbonate (1 M, 400 µL), and water (4 mL). The absorbance was measured at 760 nm after 1 hour. The calibration curve was prepared with gallic acid ranging from 0 to 1 mg mL^{-1}. The TPC were expressed as mg gallic acid per L of tea.

Antioxidant Activity Analysis

Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DDPH) radical scavenging activity assays were performed to determine antioxidant capacity

FRAP Assay

The FRAP was determined according to Benzie and Strain (1996). FRAP reagent was prepared by mixing several solutions (10:1:1) acetate buffer solution (pH = 3.6), TPTZ solution in 40 mM HCI (10 mM), and $FeCI_3$ (20 mM) solution. A total of 50-µL of the diluted sample (1:9) was mixed with 1450 µL of FRAP reagent and the absorbance was measured at 595 nm after 20 min. The results were expressed as micromoles of Trolox.

DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging activity was determined according to Brand-Williams et al. (1995). DPPH radical scavenging assay, 50-µL of the diluted sample was mixed with 1450 µL of DPPH radical solution (100 µM). The absorbance was measured at 515 nm after 60 min at room temperature.The results were expressed as micromoles of Trolox.

Statistical Analysis

The regression analysis and analysis of variance (ANOVA) were analysed ($p< 0.05$). Experimental design and data analyses were performed using the

Modde Pro (Umetrics, Sweden) software.

RESULTS and DISCUSSION

Sensorial Properties

The sensorial scores of the sage tea infusions are presented in Table 1. The scores of the colour, flavour, taste, and overall acceptability ranged from 4.3 to 5.8, from 4.4 to 5.6, from 3.1 to 4.3, and from 3.4 to 4.6.

Table 2 showed that the regression was significant ($p<0.05$) for the colour and flavour scores of the sage tea. Moreover, the quadratic models of these variables had no lack of fit value ($p>0.05$). The obtained quadratic models explained more than 90% variability in the responses ($R^2>0.90$) so they can be used to explain the effect of the studied variables on these responses.

As can be from Table 3, the most significant independent variable was temperature for the sage tea. The linear term of temperature (T) had negative impact on the colour and flavour scores of the sage tea while the quadratic term of temperature (T×T) presented significant positive impact ($p<0.10$).

Table 1. Central composite design and responses
Çizelge 1. Merkezi dizayn ve yanıtlar

Experiment no *Deney no*	Uncoded (coded) levels *Seviyeler* Temperature (°C) *Sıcaklık*	Time (min) *Süre*	Colour *Renk*	Flavour *Koku*	Taste *Tat*	Overall acceptability *Genel beğeni*	TPC (mg L^{-1}) *TFM*	DPPH (μmol) *DPPH*	FRAP (μmol) *FRAP*
1	75(-1)	2(-1)	5.24	5.35	4.10	4.57	486.40	5463.33	6399.39
2	75(-1)	6(0)	5.76	5.57	3.92	4.39	504.81	5165.00	6110.00
3	75(-1)	10(1)	5.65	5.45	3.69	4.18	457.26	5255.00	6095.86
4	85(0)	2(-1)	4.41	4.43	3.51	3.69	483.06	6403.75	7358.89
5	85(0)	6(0)	4.86	4.80	4.33	4.57	471.26	5841.67	6686.26
6	85(0)	6(0)	4.69	4.41	3.82	4.14	469.04	5633.33	6557.98
7	85(0)	10(1)	4.41	4.63	3.41	3.96	447.13	5910.00	6668.08
8	95(1)	2(-1)	4.34	4.62	3.38	3.48	581.40	5583.33	6795.86
9	95(1)	6(0)	4.40	4.70	3.50	3.84	610.05	5100.00	6367.07
10	95(1)	10(1)	4.52	4.64	3.12	3.38	531.98	5493.33	6717.58

TFM: toplam fenolik madde, DPPH: 2,2-diphenyl-1-picrylhydrazyl radikal yakalama aktivitesi, FRAP: Demir indirgeyici antioksidan güç.

Table 2. Analysis of variance (ANOVA) of sage tea
Çizelge 2. Adaçayının varyans analizi (ANOVA)

Factor *Faktör*	DF	Sum of Square *Kareler toplamı*	Mean square *Kareler ortalaması*	F-value	*p*-value
Colour *Renk*					
Regression *Regresyon*	5	2.485	0.497	17.555	**0.008**
Residual *Artık*	4	0.113	0.028		
Lack of fit *Uyum Eksikliği*	3	0.098	0.032	2.091	0.461
Pureerror *Hata*	1	0.016	0.016		
R^2: 0.96					
Flavour *Koku*					
Regression *Regresyon*	5	1.580	0.316	14.454	**0.011**
Residual *Artık*	4	0.087	0.022		
Lack of fit *Uyum Eksikliği*	3	0.011	0.004	0.046	0.981
Pureerror *Hata*	1	0.077	0.077		
R^2: 0.95					
TPC *TFM*					
Regression *Regresyon*	5	25501.800	5100.350	20.834	**0.006**
Residual *Artık*	4	979.236	244.809	133.087	0.064
Lack of fit *Uyum Eksikliği*	3	976.789	325.596		
Pureerror *Hata*	1	2.447	2.447		
R^2: 0.96					
DPPH					
Regression *Regresyon*	5	1300590.000	260118.000	11.917	**0.016**
Residual *Artık*	4	87313.800	21828.500		
Lack of fit *Uyum Eksikliği*	3	65611.100	21870.400	1.008	0.607
Pureerror *Hata*	1	21702.700	21702.700		
R^2: 0.94					
FRAP					
Regression *Regresyon*	5	1102690.000	220538.000	7.224	**0.039**
Residual *Artık*	4	122110.000	30527.400		
Lack of fit *Uyum Eksikliği*	3	113882.000	37960.600	4.614	0.327
Pureerror *Hata*	1	8227.850	8227.850		
R^2: 0.90					

Bold values are significant at 95% confidence of level. TFM: toplam fenolik madde, DPPH: 2,2-diphenyl-1-picrylhydrazyl radikal yakalama aktivitesi, FRAP: Demir indirgeyici antioksidan güç.

Table 3. Regression coefficients of sage tea
Çizelge 3. Adaçayının regrasyon katsayıları

Factor *Faktör*	**Colour** *Renk*		**Flavour** *Koku*		**TPC** *TFM*		**DPPH** *DPPH*		**FRAP** *FRAP*	
	Regression coefficient *Regrasyon katsayısı*	p-value *p-değeri*	Regression coefficient *Regrasyon katsayısı*	p-value *p-değeri*	Regression coefficient *Regrasyon katsayısı*	p-value *p-değeri*	Regression coefficient *Regrasyon Katsayısı*	p-value *p-değeri*	Regression coefficient *Regrasyon katsayısı*	p-value *p-değeri*
Mean *Ortalama*	4.718	**0.000**[a]	4.624	**0.000**[a]	481.729	**0.000**[a]	5765.220	**0.000**[a]	6654.650	**0.000**[a]
Temperature (L) *Sıcaklık*	-0.462	**0.001**[a]	-0.332	**0.003**[a]	37.780	**0.002**[a]	40.855	0.453	174.630	**0.040**[a]
Temperature (Q) *Sıcaklık*	0.283	**0.018**[a]	0.330	**0.007**[a]	42.971	**0.003**[a]	-441.092	**0.002**[a]	-301.407	**0.017**[a]
Time (L) *Süre*	0.081	0.223	0.043	0.430	-15.731	**0.039**[a]	-110.320	**0.089**[b]	-146.881	**0.065**[b]
Time (Q) *Süre*	-0.161	**0.094**[b]	-0.067	0.360	-17.960	**0.058**[b]	240.702	**0.020**[a]	213.686	**0.049**[a]
Interaction *Interaksiyon*	-0.039	0.528	-0.013	0.803	-3.414	0.548	20.185	0.703	37.777	0.552

L Linear, *Q* Quadratic, [a]Bold values are significant at 95% confidence of level, [b]Bold values are significant at 90% confidence of level. TFM: toplam fenolik madde, DPPH: 2,2-diphenyl-1-picrylhydrazyl radikal yakalama aktivitesi, FRAP: Demir indirgeyici antioksidan güç.

The colour and flavour scores of the sage tea decreased with increasing temperature until the midpoint was reached and then it changed slightly. It changed slightly with increasing time (Fig. 1 and 2).

The flavour scores of the studied herbal tea infusions were found to decrease with increasing brewing temperature, which may be attributed to their volatile compounds. *Salvia officinialis* includes α-thujone, β-thujone, 1-8-cineole, α-pinene, camphor, caryophyllene, germacrene D, viridiflorol, elemene, α-humulene, linalool, borneol, and ledene as volatile compounds (Sharifi-Rad et al., 2018). The panellists could perceive more volatile compounds with increasing temperature, leading to a less flavour score.

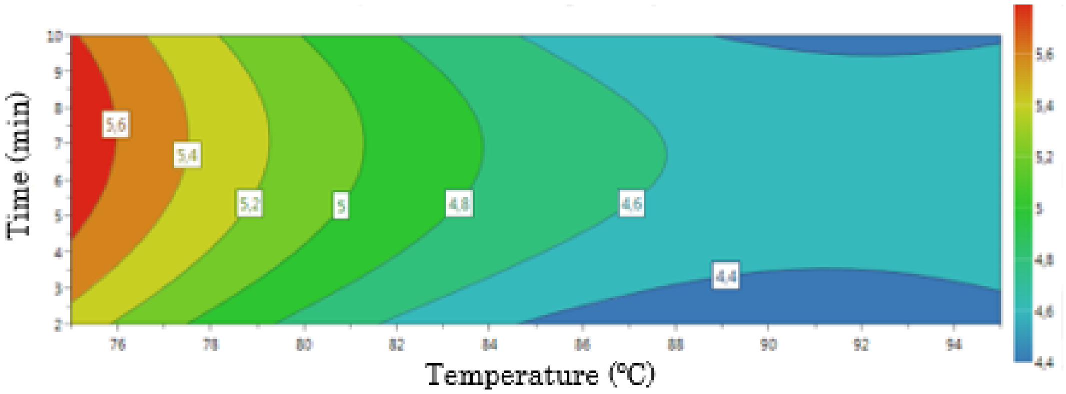

Figure1. Contour plot of colour
Şekil 1. Kontur grafiği: renk

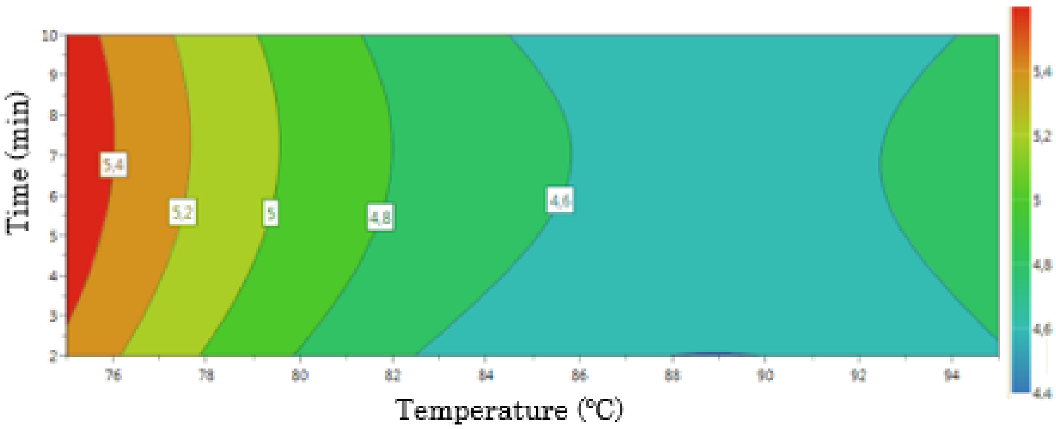

Figure 2. Contour plot of flavour
Şekil 2. Kontur grafiği: koku

Total Phenolic Content

Total phenolic content (TPC) of the herbal infusion samples is presented in Table 1. The TPC ranged from 447 to 610 mg L^{-1} for the sage tea.

Table 2 showed that the regression was significant ($p<0.05$) and the quadratic model had no lack of fit value ($p>0.05$). The quadratic model explained 96% variability (R^2 : 0.96) so it can be used to explain the effects of the studied variables on the TPC.

The regression coefficients of the generated models are shown in Table 3. The most significant independent variable was temperature for the sage tea. The linear term of temperature (T) and the quadratic of temperature (T×T) had significant positive impacts on the TPC of the sage tea ($p<0.05$) whereas the linear term of time (t) and the quadratic term of time (t×t) showed significant negative impacts on the TPC ($p<0.10$).

The TPC of the sage tea increased with increasing temperature after the midpoint was reached. It decreased with increasing time after the midpoint was reached (Fig. 3).

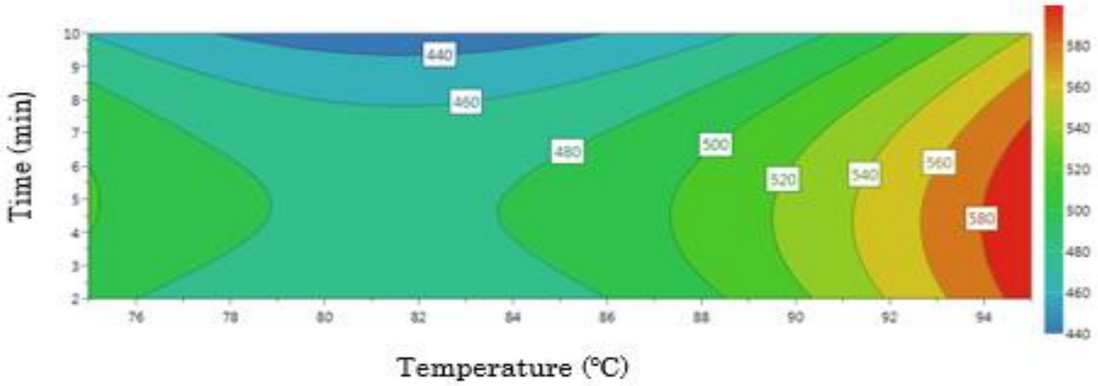

Figure 3. Contour plot of TPC
Şekil 3. Kontur grafiği: TFM

The TPC of the sage tea presented an increasing trend between 83 °C and 95°C. According to Sun et al. (2017), the TPC of the wolfberry infusion showed an increased trend with increasing temperature, ranging from 60 °C to 100 °C. Komes et al. (2010) found that the green tea samples reached their maximum TPC at 100 °C when they were brewed at 60 °C, 80 °C, and 100 °C. Dent et al. (2012) reported that the TPC of sage's water extract increased at higher temperatures from 60°C to 90°C. A higher temperature may improve the solubility of phenolic compounds and enhance the permeability of cell walls, increasing the diffusion coefficients of phenolic compounds, which may explain the results of this study (Harbourne et al., 2013).

The TPC of the sage tea decreased with increasing time between 6 and 10 min, which could be attributed to the decomposition of phenolic compounds with a longer infusion time (Sentkowska et al., 2016).

DPPH Radical Scavenging Activity

The DPPH values of the tea samples are presented in Table 1. The DPPH value ranged from 5100 to 6404 µmol TE for the sage tea.

Table 2 showed that the regression was significant ($p<0.05$) and the generated model had no lack of fit value ($p>0.05$). The model explained 94% variability in the DPPH value (R^2: 0.94), so it can be used to explain the effects of the studied variables on the DPPH radical scavenging activity.

The regression coefficients of the generated models are shown in Table 3. The most significant independent variable was time. It can be seen from Table 3, the quadratic term of time (t×t) had significant positive impact on the DPPH value of the sage tea whereas the linear term of time (t) and the quadratic of temperature (T×T) showed significant negative impacts on the DPPH value ($p<0.10$).

The DPPH value of the sage tea increased with increasing temperature until the midpoint was reached and then it decreased. It decreased with increasing time until the midpoint was reached (Fig. 4).

Similar findings were found in the literature. Sotiropoulou et al (2020) found that the antioxidant capacity of sage extract increased from 25 to 80 °C and decreased between from 80 to 100 °C. The antioxidant activity of the herbal tea extracts such as sage, chamomile, linden, lingia and gyokuro were determined to decrease at the highest temperature (Horžic et al., 2009; Stagos et al., 2012; Cvetanovic et al., 2019). A reduction in the DPPH value of the sage tea was observed after it increased up to the certain extent, which may be related to the degradation of antioxidant compounds or alterations in their molecular structure with a higher infusion temperature. A reduction in the antioxidant capacity was previously attributed to a loss of antioxidant compounds due to intense thermal treatment (Sotiropoulou et al., 2020). Furthermore, the polymerization of phenolic compounds may reduce antioxidant activity (Fu et al., 2018).

The DPPH value of the sage tea decreased with increasing infusion time. The decomposition of antioxidant compounds could lead to a reduction in the DPPH radical scavenging activity.

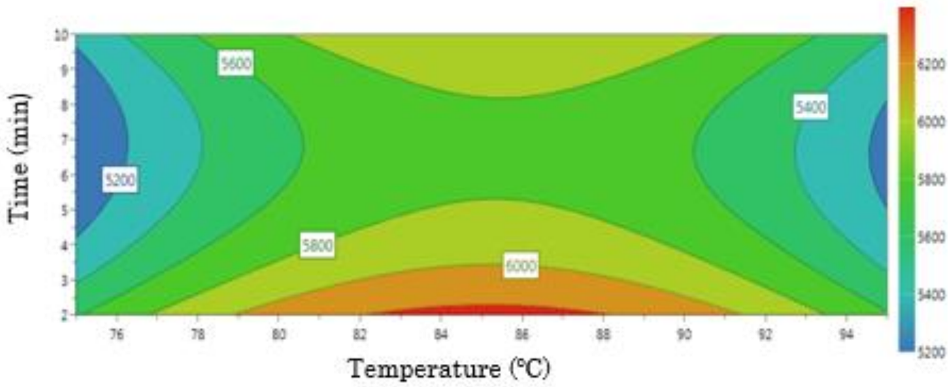

Figure 4. Contour plot of DPPH
Şekil 4. Kontur grafiği: DPPH

FRAP

The FRAP values of the tea samples are presented in Table 1. The FRAP value ranged 6110 to 7359 µmol TE for the sage tea.

Table 2 showed that the regression was significant ($p<0.05$) and the generated model had no lack of fit value ($p>0.05$). The model explained 90% variability in the FRAP value (R^2 : 0.90) so it can be used to explain the effects of the studied variables on the FRAP.

The regression coefficients of the generated models are shown in Table 4. The most significant independent variable was temperature for the sage tea. The linear term of temperature (T) and the quadratic term of time (t×t) had significant positive impacts on the FRAP value of the sage tea ($p<0.05$) whereas the quadratic term of temperature (T×T) and the linear term of time (t) presented significant negative impacts on the FRAP value ($p<0.10$).

The FRAP value of the sage tea increased with increasing temperature until the midpoint was reached and then it changed slightly. The FRAP value of the sage tea decreased with increasing time until the midpoint was reached and then it changed slightly (Fig. 5).

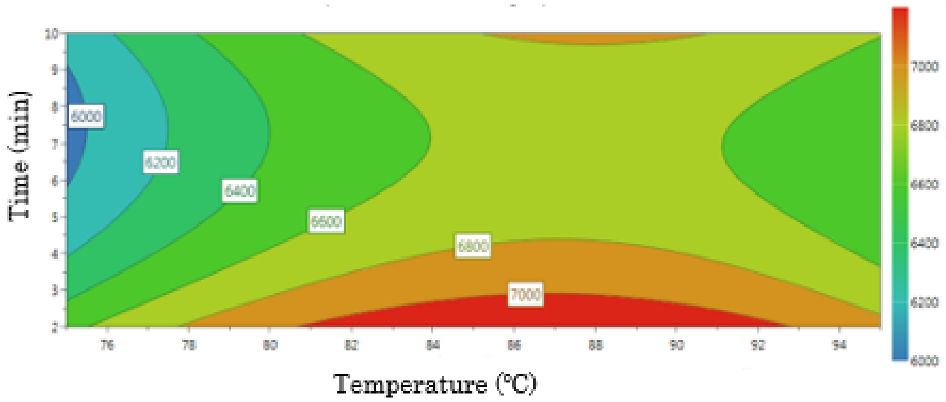

Figure 5. Contour plot of FRAP
Şekil 5. Kontur grafiği: FRAP

The FRAP value of the sage tea increased to reach the midpoint of the response surface, and then there was no further increase. Similar results were reported for black tea. Black tea showed a linear increase between 20 °C and 70 °C, and with no further increase between 70 °C and 90 °C (Langley-Evans, 2000). With increasing infusion temperature, an increase in the TPC could be resulted in an increase in the antioxidant activity.

The FRAP value of the sage tea decreased with increasing infusion time, which may be related to the decomposition of antioxidant compounds (Sentkowska et al., 2016).

CONCLUSION

The results of this study revealed that the infusion temperature had negative effect on the sensorial properties and infusion time had negative effect on the antioxidant capacity at the studied brewing conditions (75-95 ℃ and 2-10 minutes). It can be concluded that the best brewing conditions were the lowest water temperature (75-80ºC) and the shortest brewing time (2-4 min) for the studied brewing conditions. Lower infusion temperature and shorter infusion time can be recommended to obtain the most admired sage tea infusion with higher antioxidant capacity.

Author Contribution

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

Conflict of Interest

The authors declare that there is no conflict interest

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