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Response Surface Optimization of Extraction Parameters of Green Tea

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ABSTRACT

Green tea is one of the most popular beverage in the world. Green tea (*Camellia sinensis*) is a rich source of bioactive components so it has been used in traditional medicine. In the present study, green tea is extracted with water to obtain maximum nutritional quality and antioxidant activity. The optimal conditions for extraction of green tea was determined by Response Surface Methodology (RSM). Box-Behnken design was applied to evaluate the effects of three independent factors, leaf to water ratio (1:10, 1:30 1:50), temperature (40 to 50°C) and extraction time (15 to 45 min). Optimal conditions were, extraction temperature of 51.44°C, time 29.48 min and water to solid ratio of 1:47.80 with highest total polyphenols, total flavonoid, caffeine, antioxidant activity and lowest tannin.

Highlights

- Study gives optimum conditions for extraction functional components
- Green tea is good source of flavonoids and polyphenols
- Study shows that medicinal value of green tea is due to its exhalent antioxidant capacity

Keywords: Green tea, antioxidant activity, flavonoids, caffeine, total phenols

Tea is the second most popular drink in the world after water. Tea can be classified into three major categories: unfermented green tea, partially fermented oolong tea, and fermented black tea (Yu *el al.,* 2014). Green tea contains more catechins, than black tea or oolong tea. So it is included in the group of beverages with functional properties (Sinija and Mishra 2008). Green tea is steamed and heated immidiatly after harvesting to stop the enzymatic activity in order to retain the majority of catechin and flavonoids (Ozturk *el al.,* 2016).

Green tea is getting popular recently due to increasing awareness of its health benefits. Studies indicate a wide variety of health benefits, its regular consumption reduced risk of cardiovascular disease and certain types of cancer, in- flammatory bowel, liver and neurodegenerative diseases, diabetes, and even weight loss. These health benefits are attributed to its high content of catechins which have been described as potent antioxidants ameliorating disease states related to reactive oxygen species (Carloni *el al.,* 2013).

Green tea beverage is made be infusing the green tea leaves in hot water, quality of tea infusion is influenced by presence of polyphenols, amino acids, saccharides, flavonoids and caffeine. The water extraction method is excellent because of its high yield rate, low cost and safety (Danrong *el al.,* 2009) hence water is selected as the extraction media in our study. The present study is aimed at the optimization process parameters for extraction of functional components from green tea.

MATERIALS AND METHODS

Materials

Green tea was purchased from Kanan Devan Hills Plantations Company (P) Ltd. Kerala, India. Standards of caffeine, catechin and 2, 4 ,6- tripyridyl-s- triazine was purchased from Sigma Aldrich. All other chemicals used were of analytical grade.

Green tea leaves were weighed accurately and extracted with water in three different temperature, time and water ratio. Extract was analysed for total polyphenols, total flavonoids, caffeine, tannins and antioxidant activity.

Total polyphenols

Total phenolics were determined colorimetrically using Folin-Ciocalteau reagent and concentrations are expressed as gram of gallic acid equivalents per one g of dry weight (Hajimahmoodi *et al.*, 2008).

Total flavonoids

Total flvonoid content was measured by the aluminum chloride colorimetric assay. Total flavonoid content was expressed as mg catechin equivalents/g dry mass (Hajimahmoodi *el al.,* 2008).

Caffeine

Caffeine Estimation was carried out according to method described by Sinija and Mishra 2009. Absorbance of different samples was measured at 277 nm against pure chloroform as blank using UV–VIS spectrophotometer.

Tannins

Estimation of tannin was performed by titrating sample with standard potassium permanganate solution (AOAC, 1980).

Antioxidant activity

The FRAP (Ferric reducing antioxidant power assay) procedure was used to estimate antioxidant activity and it is expressed in mmol Fe per gram (Yashin, *el al.,* 2011).

Statistical analysis

RSM was used to investigate the main effects of process variables on total polyphenols, total flavonoids, caffeine, tannins and antioxidant activity. The coded and uncoded independent variables used in the RSM design were listed in Table 1.

Table 1: Uncoded and coded independent variables used in RSM design

Code	Independent variable	Coded levels		
		-1	0	+1
A	Leaf-water ratio	1:10	1:30	1:50
B	Time (min)	15	30	45
C	Temperature (°C)	40	50	60

A 17-run Box–Behnken design with three factors and three levels, including five replicates at the centre point, experimental points used according to this design are shown in Table 2. Statistical analysis was done with the aid of the Design-Expert software version 7.0.0.

RESULTS AND DISCUSSION

Results of different runs of extraction were shown in Table 2. Regression equation coefficients of the proposed models and statistical significance of all main effects calculated for each response were obtained and effects being not significant ($p > 0.05$) were stepped down from models without damaging the model hierarchy. ANOVA showed that the lack of fit was not significant for total flavonoids and significant for other response.

Total polyphenols

The experimental data for extractable total polyphenols is given in table 2. The effects of material to water ratio, extraction time and temperature on extractable polyhenols, as well as their interactions, are shown in Fig. 1. Total polyphenols was ranges from 3.87 to 12.56 mg/g and maximum polyphenols was extracted 1:30, 30 min and 60°C. Total polyphenols increased significantly with increase in water ratio, while the total polyphenols extraction reached a maximum when extraction time was up to a certain value, without significant further improvement thereafter (Fig. 1). Increasing temperature tended to reduce the total amount of catechins which are main component of tea polyphenols, it was concluded that oxidation occurred during heating besides the epimerization, this oxidation changed the tea liquor

Table 2: Box-Behnkan design with independent and response variables

Run	Water	Time (min)	Temperature (°C)	Polyphenols (mg/g)	Flavonoids (mg/g)	Caffeine (mg/g)	Antioxidant activity (mM/g)	Tannins (%)
1	10	15	50	3.789	12.500	6.750	1.079	1.181
2	50	15	50	9.972	17.000	13.500	1.968	2.300
3	10	45	50	3.875	15.000	7.150	1.180	1.089
4	50	45	50	11.010	25.000	16.950	2.499	3.625
5	10	30	40	5.495	14.000	4.950	1.025	1.390
6	50	30	40	10.195	20.500	12.350	2.267	2.778
7	10	30	60	6.215	18.000	15.870	1.395	1.792
8	50	30	60	12.560	26.500	23.750	2.464	3.964
9	30	15	40	7.357	13.000	5.500	1.343	1.635
10	30	45	40	9.294	15.500	7.950	1.535	2.147
11	30	15	60	8.790	17.000	15.250	1.698	2.475
12	30	45	60	11.380	21.500	17.750	1.870	3.798
13	30	30	50	9.152	23.000	12.000	1.615	2.042
14	30	30	50	9.827	23.400	16.980	1.734	2.192
15	30	30	50	9.923	23.900	16.950	1.751	2.213
16	30	30	50	9.249	23.300	16.920	1.632	2.063
17	30	30	50	10.019	23.800	16.970	1.768	2.235

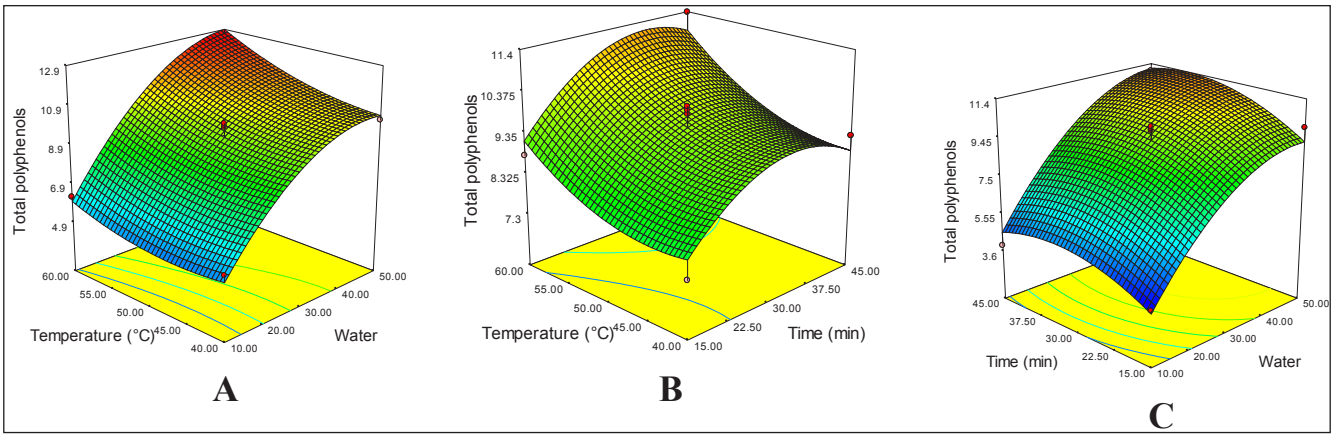

Fig. 1: Response surface plots for total polyphenols at different condition

color to be darker and less green (Ananingsih *el al.*, 2013). Similar result was observed by Hilal and Engelhardt 2007 for green tea (13.7–24.7 g/100g) and Hajimahmoodi *el al.*, 2008 for Ahmad green tea.

For total polyphenols the coefficient estimates and the corresponding P-values suggest that, among the test variables used in the study, B (Time), C (Temperature), A^2(leaf to water ratio^2), B^2(Time^2) were significant model terms with P- values of less than and 0.05. A (leaf to water ratio) ($P < 0.001$) had largest effect on total polyphenols. The mathematical equation expressing relationship of total polyphenols with variables A, B and B is given in table 3 in terms of coded factors. The p-value of the model was 0.0001 (Table 4), which indicated that the model fitness was significant. By analysis of variance, the R^2 value of this model was determined to be 0.9730, which showed that the regression model defined well the true behavior of the system.

Total flavonoids

It arised from the results of statistical analysis that total flavonoids extraction depended on the extraction time, temperature and amount of water and it ranges from 12.5 to 26.5 mg Catechin/g of green tea. Obtained value of total falvonoids is similar to

Table 3: RSM models and coefficient of determination (R^2) values

Molecule	RSM model	R^2
Total polyphenols	= 9.63 + 3.05(A) + 0.71(B) + 0.83(C) + 0.24(A*B) + 0.41(A*C) + 0.16(B*C) -1.53(A^2) – 0.94(B^2) +0.51(C^2)	0.9730
Total flavonoids	= 23.48 + 3.69(A) + 2.19(B) + 2.50(C) + 1.37(A*B) + 0.50 (A*C) + 0.5(B*C) - 1.55(A^2) -4.55(B^2) - 2.18(C^2)	0.9937
Caffeine	= 15.96 + 3.98(A) + 1.10(B) + 5.23 (C) + 0.76(A*B) + 0.12(A*C) + 0.012(B*C) - 1.13(A^2) -3.75(B^2) - 0.60(C^2)	0.9533
Antioxidant activity	= 1.70 + 0.56(A) + 0.12(B) + 0.16(C) + 0.11(A*B) - 0.043(A*C) – 5.175E-003(B*C) + 0.079(A^2) - 0.098(B^2) + 9.050E-003(C^2)	0.9893
Tannins	= 2.15 + 0.90(A) + 0.38(B) + 0.51(C) + 0.35(A*B) + 0.20(A*C) + 0.20(B*C) - 0.067(A^2) - 0.034(B^2) + 0.40 (C^2)	0.9842

Table 4: ANOVA of RSM models

Molecule	Variation source	SS	DF	MS	F value	Prob-F
Total polyphenols	Model	99.52	9	11.06	27.98	0.0001
	Residual	2.77	7	0.40		
	Lack of fit	2.12	3	0.71	4.34	0.0949
	Pure error	0.65	4	0.16		
	Total	102.29	16			
Flavonoids (mg/g)	Model	334.27	9	37.14	123.19	< 0.0001
	Residual	2.11	7	0.30		
	Lack of fit	1.56	3	0.52	3.80	0.1149
	Pure error	0.55	4	0.14		
	Total	336.38	16			
Caffeine (mg/g)	Model	427.64	9	47.52	15.87	0.0007
	Residual	20.96	7	2.99		
	Lack of fit	1.31	3	0.44	0.089	0.9623
	Pure error	19.64	4	4.91		
	Total	448.59	16			
Antioxidant activity (mM/g)	Model	2.99	9	0.33	71.78	< 0.0001
	Residual	0.032	7			
				4.631E-003		
	Lack of fit	0.012	3	4.063E-003	0.80	0.5536
	Pure error	0.020	4	5.057E-003		
	Total	3.02	16			
Tannins (%)	Model	11.05	9	1.12	48.48	< 0.0001
	Residual	0.18	7	0.026		
	Lack of fit	0.15	3	0.049	6.12	0.0563
	Pure error	0.032	4	8.082E-003		
	Total	14.44	16			

findings of Hajimahmoodi *el al.*, (2008) who reported total flavonoids of 22.752 mg Catechin/g Chinas green tea. Total flavonoids extraction increases with increase in time, temperature and amount of water and it is maximum at when extraction time is 30 min, temperature is 60°C and water is 50 times and further increase in extraction time gave slight or no increase in total flavonoids (Fig. 2). This was explained by Labbe *el al.*, 2006, EGC and EC which are time dependent components and solubilisation of these components increased rapidly between 0 and 20 min and after stabilized, and whatever the brewing temperature, this study reported extraction of green tea catechins was maximum at 50°C for 20-40min. Similar trend was observed by Vuong *el al.*, (2011) for extraction of green tea catechins

(water-to-tea ratio of 20:1 time 30 min).

The magnitude of p-value indicates that all linear terms (A, B, C), quadratic terms B^2 and C^2 were highly significant 1 per cent level ($p < 0.0001$), interaction term A*B quadratic terms A^2 were significant 5 percent level ($p < 0.05$). But interaction term B*C and A*C had not significant at 5 percent level ($p > 005$). The p-value (Table 4), of the model was <0.0001. The best fit model was expressed by the coefficient of determination R^2, which was0.9937 (Table 3), indicating that 99.37 per cent of the variability of the response could be explained by the model. The quadratic model for total polyphenols in terms of coded levels of the variables is given in Table 3.

Caffeine

Caffeine content of extract ranged from 4.95 to 23.75 mg/g. With the increase in leaf to water ratio and temperature there is significant increase in concentration caffeine (Fig. 3). According to the report of Ziaedini *el al.*, 2010, temperature has a positive effect on extraction efficiencies and rates when it is not too high and they reported caffeine extraction was maximum at 20-40 min. Highest caffeine was extracted in 1:50 leaf-water ratio at 60 °C for extraction time of 30 min. Similar trend was observed by Labbe *el al.*, (2006) for extraction green tea caffeine and they reported caffeine solubilisation increased with a rise in brewing temperature and increased in a moderate manner with infusion duration and reported best condition as 70–80°C and 20–40 min.

For caffeine the coefficient estimates and the corresponding P-values suggest that, among the test variables used in the study, A (leaf to water ratio) and B^2 (Time^2), were significant model terms with P- values of less than and 0.05 and C (Temperature)

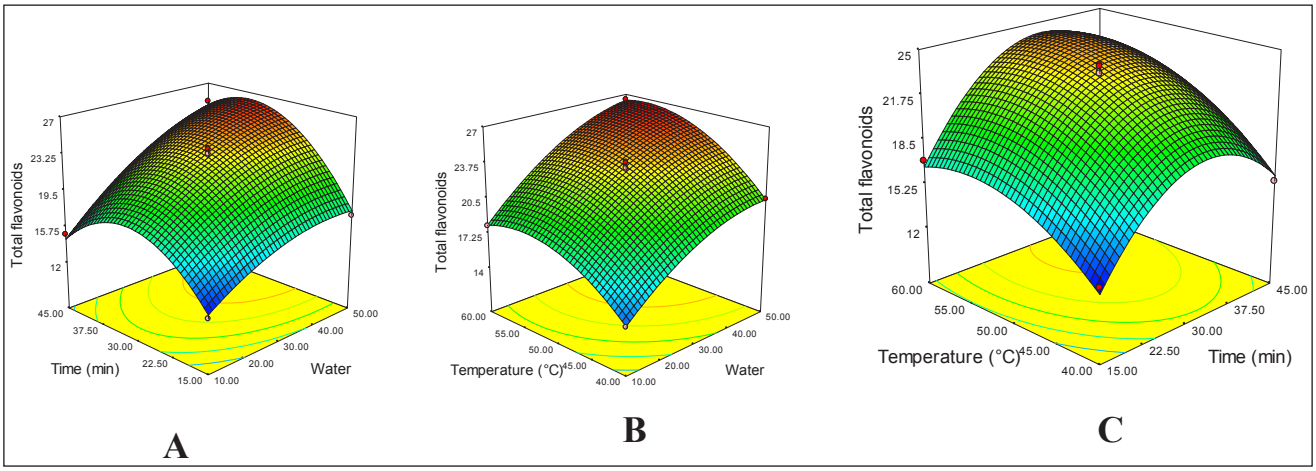

Fig. 2: Response surface plots showing effect of independent parameter on total flavonoids

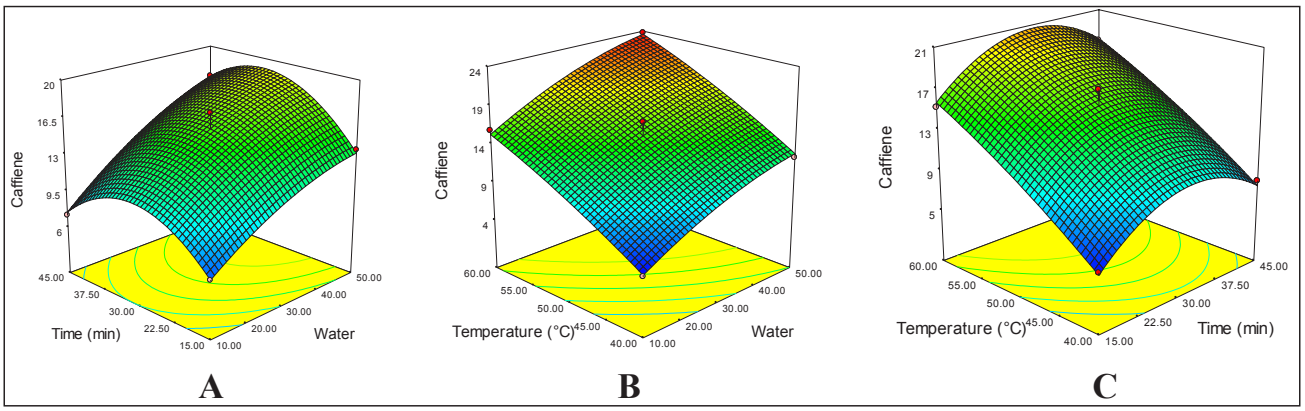

Fig. 3: Response surface plots showing influence of process parameter on Caffiene

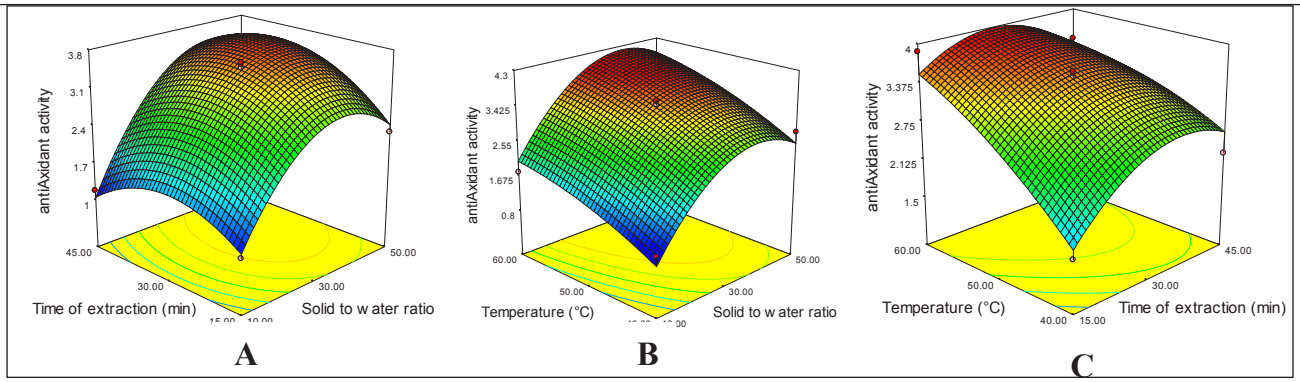

Fig. 4: Response surface plots showing effect of leaf-water ratio, time and temperature on antioxidant activity

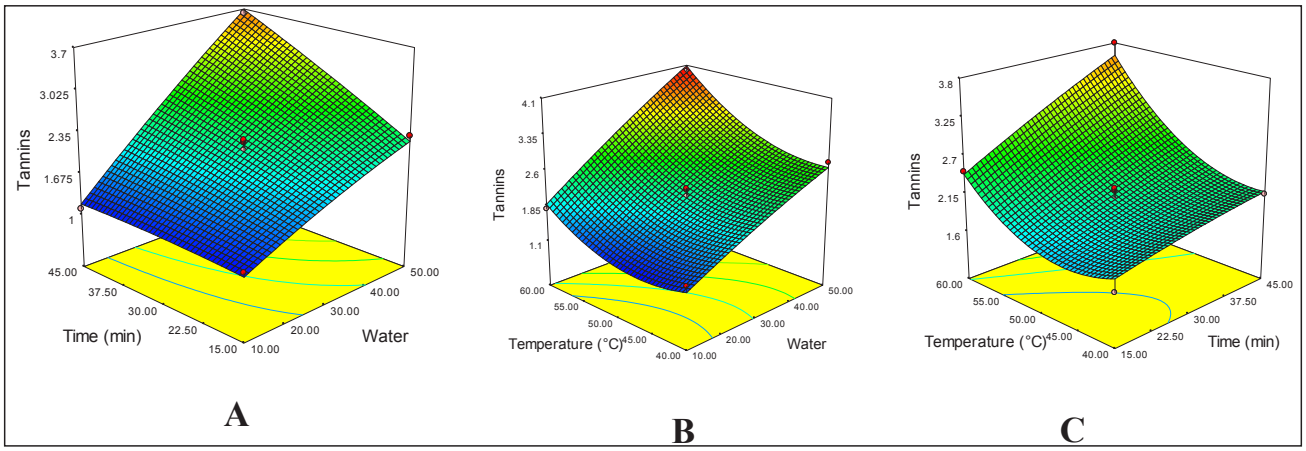

Fig. 5: Response surface plots showing influence of process parameter on tannins

was highly significant model terms with P- values of less than and 0.0001. The mathematical equation expressing relationship of caffeine with variables A, B and B is given in Table 3 in terms of coded factors. The p-value of the model was 0.0007, which indicated that the model fitness was significant. By analysis of variance, the R^2 value of this model was determined to be 0.9533, which showed that the regression model defined well the true behaviour of the system.

Antioxidant activity

Antioxidant activity of tea extracted in different condition was ranged from 1.025-2.464 mM/g. Antioxidant activity of tea extract increase with increment in all three parameters, highest value (2.464 mM/g) was recorded in 1:50 leaf-water ratio, 30 min of infusion time at 60°C. High catechin levels have been positively correlated with tea radical scavenging properties (Tenore *el al.*, 2015) that might be the reason for high antioxidant activity at high temperature and time. Similar value of antioxidant value of green tea is reported in other studies Yashin *el al.*, 2011, Hajimahmoodi *el al.*, 2008 and Gimenez *el al.*, 2013. Response surface generated for antioxidant activity as the function of two independent variables given in Fig 4. All linear coefficients (A, B and C), two quadratic coefficients (A^2 and B^2) and interaction factor of water and time had significant effect on antioxidant activity and among the six significant terms leaf-water ratio was highly significant with p value less than 0.0001. The mathematical model representing the antioxidant activity of green tea extract as a function of the independent variables within the region under investigation was expressed in equation (Table 3).

The value of the determination coefficient for the equation for tea antioxidant activity is R^2 0.9893, which indicates that only 2% of the total variation is not explained by the model. The p-value of the model was less than 0.0001, which indicated that the model fitness was significant.

Tannins

Tea tannins are soluble in water and responsible for the typical bitter taste of tea. Extraction parameters have significant affect on tannins. Minimum tannins value (1.089%) was obtained at the lowest extraction time, temperature and water ratio and it reached maximum (3.964%) value at 60°C, 30min and 1:50 water ratio, similar treads is observed by Mache *el al.*, 2015 for Khaya tea tannins and they reported with increment in water volume, infusion time and temperature there will be increase in tannin extraction. Obtained values are in line with the findings of Kopjar *el al.*, 2015 and Khasnabis *el al.*, 2015 for commercially available green tea (2.65%). According to literature tannin decrease feed intake, growth rate, feed efficiency and protein digestibility. Tannins are known to bind to dietary iron and prevent its absorption specifically of 'nonheme' iron found in plant foods (Khasnabis *el al.*, 2015), so tea with less tannin content is preferable. Effect of two independent factors on tannins is given in Fig. 5.

ANOVA (Table 4) was performed to evaluate the significance of the coefficients of the quadratic polynomial models. On the basis of the regression coefficients and the p-value, it was found that the linear (B), quadratic (C^2) and all interaction terms had significant effects effect on tannin content ($p<0.005$). The variable with the greatest effect is linear terms of time (A) and temperature (C) with $p<0.0001$.

Regression equations describing the mathematical relationships between the independent and response variables obtained (Table 3). The p-value of the model was <0.0001, which indicated that the model fitness was significant. The model can fit well with the actual data when R^2 approaches unity. By analysis of variance, the R^2 value of this model was determined to be 0.9842.

Optimization

Optimum conditions for extraction of green tea components were determined to obtain the criteria; maximum total polyphenols, total flavonoid, caffeine, antioxidant activity and minimum tannins content. By applying desirability function method, solutions were obtained for the optimum extraction criteria with desirability value of 0.709.

CONCLUSION

In this study, process parameter for extraction of functional components from green tea was optimized. Optimization responses were total polyphenols, total flavonoid, caffeine, antioxidant activity and tannins. By RSM and desirability value optimum condition for maximum functional component in green tea extract is 1:47.80 leaf-water ratio, 29.48 min extraction time and 51.44°C extraction temperature to obatain total polyphenols of 11.282 mg/g, total flavonoids of 25.787 mg/g, caffiene content of 19.310 mg/g, antioxidant activity of 2.275 mM/g and tannins 2.982%.

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