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Optimization of brewing conditions in epigallocatechin-3-gallate (EGCG) extraction from Jinxuan summer green tea by response surface methodology

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Summary

The extraction conditions of epigallocatechin-3-gallate (EGCG) from Jinxuan summer green tea and antitumor activity against human gastric cancer SGC-7901 cells of the green tea extracts were investigated. On the basis of a single factor experiment, Box-Behnken design and response surface methodology were employed to optimize the hot water extraction conditions. The optimal extraction conditions for EGCG were determined as: extraction temperature of 85 °C, extraction time of 34 min, water-tea ratio of 41 mL/g, a solution of pH 6, and extraction twice. Under these conditions, the experimental extraction yield value of EGCG was 33.82 mg/g, which was not significantly different in comparison to predicted values. The results indicated that the regression models were suitable for the EGCG extraction from Jinxuan summer green tea. The summer green tea extract prepared under the optimal conditions had a higher antitumor activity against human gastric cancer SGC-7901 cells than the green tea extract made with traditional tea brewing method.

Keywords: Response surface methodology, Summer green tea, EGCG, Gastric cancer

Introduction

Camellia sinensis tea is the second most widely consumed non-alcoholic beverage in the world (Dalar and Konczak, 2013). Tea is usually classified into unfermented (green tea), slight-fermented (white tea), semi-fermented (oolong tea), fermented (black tea), and post-fermented (dark green tea) forms (Zhao et al., 2014). Among them, green tea is one of the most popular beverages consumed worldwide. And its consumption is increasing as a result of accumulated scientific evidence on its beneficial effects for human health. The epidemiological studies have suggested that daily consumption of green tea will help to prevent cancer, cardiovascular diseases, dental decay, obesity, diabetes, and improve the immune system (Kao et al., 2006; Khan and Mukhtar, 2007; Vuong et al., 2013; Wheeler and Wheeler, 2004). The aforementioned health benefits of green tea have been closely associated with tea catechins, purine alkaloids and theanine. Tea catechins which usually account for 30% of the dry weight of the green tea are associated with various health benefits, including antioxidant activity, antitumor activity, and anti-aging activity (Khan and Mukhtar, 2007; Savic et al., 2016).

Epigallocatechin-3-gallate (EGCG) has been considered as the most abundant and active constituent in green tea and may account for up to 50% of the catechins. It is usually used as a quality indicator (Gadkari et al., 2015; Khan and Mukhtar, 2007; Perva-Uzunalić et al., 2006). A number of studies have reported that EGCG has various biological, physiological, and pharmaceutical activities, such as prevention of obesity, hyperglycemia, insulin resistance, hypercholesterolemia, and hepatic steatosis (Chen et al., 2011; Hininger-Favier et al., 2009; Liu et al., 2012; Sae-tan et al., 2011). Additionally, it has been reported to have excellent anticancer and antioxidant activities (Garbisa et al., 2001; Singh et al., 2011; Xu et al., 2010). Hence, EGCG may be extensively applied in the fields of medication, cosmetics, beverages, and foodstuffs (Pavlovic et al., 2015).

Owing to its great potential as a health promoter, the extraction of EGCG from green tea is considered to be an important research work for its wide use in a variety of health and food products (Zhang et al., 2012). The extraction efficiency of the tea constituents and quality of obtained extracts have been directly influenced by several extraction conditions, including brewing temperature, extraction time, the ratio of solvent to tea, tea particle size, the pH of the brewing solution and the number of times the same sample is extracted (Castiglioni et al., 2015; Hajiaghaalipour et al., 2016; Saklar et al., 2015). Thus, the extraction efficiency can be improved by optimizing each of these factors during the extraction process. The conventional single factor experiment for performing optimization is very time-consuming and the interactions among multiple parameters are not considered in this method (D'Archivio et al., 2016). Response surface methodology (RSM) is a helpful statistical technique which is useful for developing, improving and optimizing processes. It can minimize the number of experiments and provide sufficient information for a statistically acceptable result. RSM has become more and more attractive in process optimization, which has been successfully applied in the extraction of biologically active constituents from plant sources (Kong et al., 2010; Savic et al., 2015; Savić et al., 2013).

Therefore, the aim of the present study was to extract EGCG using the RSM with a Box-Behnken design (BBD) to optimize the extraction conditions and obtain maximum EGCG from summer green tea (the lower grade green tea). According to the results of a single factor experiment, extraction temperature, extraction time and water-to-tea ratio were selected as independent variables for a Box-Behnken design. Meanwhile, the number of times the tea is extracted and the pH of the brewing solution were set as fixed values. The chemical compositions of the lower grade green tea extract was analyzed, identified and quantified using an HPLC (high performance liquid chromatography) gradient type elution system and authentic standards. Additionally, the lower grade green tea extract was tested *in vitro* for its antitumor activity against human gastric cancer cells.

Materials and methods

Materials

The summer green tea of the *Jinxuan* variety (*Camellia sinensis*), harvested in June 2013, was kindly provided by Guangdong Minghuang Tea Co., Ltd. (Guangdong, China). The dried green tea was ground by a disintegrator of FW100 (Taisite Co., Ltd, Tianjin, China) and then passed through a 40-mesh sieve. The tea power was kept in airtight bags at -20 °C until use. The conventional chemical composi-

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tions of green tea used in brewing trails were determined according to the GB/T of Chinese standard (GB/T 8313-2008 and GB/T 8314-2013). The data analysis for the green tea used in brewing trails is shown in Tab. 1. Deionized water was prepared using a Millipore ZMQS5001 system (Millipore, Bedford, MA, USA). Standards of C (catechin), CG (catechin gallate), EC (epicatechin), ECG (epicatechin gallate), EGC (epigallocatechin), EGCG (epigallocatechin-3-gallate), GC (gallocatechin), GCG (gallocatechin gallate) and caffeine were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents used for HPLC analysis were HPLC grade and were purchased from Thermo Scientific (Waltham, MA, USA). All other reagents were of analytical grade.

Tab. 1: The composition of green tea leaves (g/100 g dry matter).

Green tea	g/100 g dry matter*
Tea polyphenols	27.33 ± 0.25
Amino acids	4.58 ± 0.05
Caffeine	4.74 ± 0.07
Soluble sugars	3.42 ± 0.04
Soluble protein	0.64 ± 0.01

* Values are mean ± standard deviation of three measurements

EGCG extraction

Three grams of pretreated green tea powder was extracted with distilled water using a designed temperature, time, brewing solution pH, water-tea ratio and the extraction times in a HHS thermostat water-bath (Aohua Co., Ltd, Changzhou, China). Following extraction, the mixtures were centrifuged at 8000 rpm for 10 min by the centrifuge (HC-3018, Anhui USTC Zonkia Scientific Instruments Co., Ltd, Anhui, China). The supernatant was collected and transferred into a 250 mL measuring flask for determination of EGCG content. Each extraction was performed in three replicates. Samples (the extracts) were stored at -20 °C until HPLC analysis.

Single-factor design for EGCG extraction

The extraction yield of EGCG depends on the factors, including temperature, extraction time, brewing solution pH, water-tea ratio and the extraction times. The single-factor design was used to determine the preliminary range of extraction factors. Single factor experiment was used to study the effect of different factors on the extraction yield of EGCG.

For single factor experiment, one factor was changed in a certain range while all other factors were kept constant. The extraction parameters were temperature (50, 60, 70, 80 and 90 °C), time (10, 20, 30, 40 and 50 min), water-tea ratio (10, 20, 30, 40 and 50 mL/g), times (once, twice, thrice, and four times), and brewing solution pH (4, 5, 6, 7 and 8), of which the single factor experiment was investigated in this paper, respectively.

Response surface optimization designs

According to the results of single-factor experiment, three major factors and their appropriate ranges were finally determined for a three-level factorial Box-Behnken design (BBD) in order to investigate the relationship between process variables and the extraction yield of EGCG as the response value (Tab. 2). The whole design comprising 17 experimental runs were carried out in a randomized order as shown in Tab. 3. The following second-order polynomial equation was used to explain the behavior of the system:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

where Y represents the predicted extraction yield of EGCG, X_i and X_j represent the independent variables. β_0, β_i, β_{ii} and β_{ij} represent the regression coefficient for intercept, linearity, quadratic and interactive terms, respectively.

The data were analyzed using Design Expert program (7.0.0 version, Stat-Ease Inc., Minneapolis, Minnesota, USA). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design.

Tab. 2: The uncoded and coded levels of independent variables used in the RSM design.

		Levels		
Independent variable	Symbol	Low (-1)	Middle (0)	High (+1)
Temperature (°C)	X_1	50	70	90
Time (min)	X_2	20	40	60
Water-tea ratio (mL/g)	X_3	10	30	50

Determination of tea catechins content

The tea catechins in extract were quantified by HPLC using an Agilent 1200 series HPLC system equipped with a UV-detector and an analytical workstation (Agilent Co., Ltd, Santa Clara, CA, USA). The separation was performed on an Agilent ZORBAX EclipseXDB-C_{18} column (4.6 mm × 150 mm, 5 μm; Agilent Technologies, Wilmington, DE, USA). The solvents used were: (A) acetonitrile/acetic acid/methanol/water (1:0.5:2:96.5, v/v/v/v), and (B) acetonitrile/acetic acid/methanol/water (10:0.5:20:69.5, v/v/v/v). The following linear gradient elution was used: 0-30 min, A from 72.5% to 20%; 30-35 min, A from 20% to 72.5%. The column temperature was 28 °C. The flow rate was 1 mL/min. The detection wavelength was 280 nm and the injection volume was 10 μL. Quantification was carried out with the external standard method. Calibration plot was constructed by tea catechins concentrations versus each plotting peak areas. The concentrations of tea catechins in extracted samples were obtained by linear calibration curve.

Determination of antitumor activity of green tea extracts

Preparation of green tea extracts for cell viability assay

In order to evaluate the antitumor activity of green tea infusions brewed with different methods, traditional tea brewing method (TTBM) and improved tea brewing method (ITBM) were chose to prepare tea infusion. Different brewing methods to prepare tea infusion are mentioned below:

(1) Traditional tea brewing method (TTBM): tea infusions were prepared by placing 1 g of tea in 50 mL of distilled water at boiling temperature (100 °C) twice, and 3 min each time.

(2) Improved tea brewing method (ITBM): tea infusions were prepared by placing 1 g of tea in 41 mL of distilled water (pH 6) at 85 °C twice, and 34 min each time.

The tea infusions were freeze-dried. The concentrated green tea extracts were stored at -20 °C for assay.

Cell viability assay

Human gastric cancer cell SGC-7901 was obtained from Shanghai Institute of Cell Biology (Shanghai, China) and maintained in an atmosphere of 5% CO_2 and 95% air at 37 °C in DMEM medium supplemented with 10% fetal bovine serum (FBS, Gibco/BRL, Gaithersburg, MD, USA). Cell viability assay was determined by

Tab. 3: Box-Behnken design of factors with experimental and predicted values.

				EGCG content (mg/g)	
Run	X_1Temperature (°C)	X_2Time (min)	X_3Water-tea ratio(mL/g)	Experimental	Predicted
1	70 (0)	40 (0)	30 (0)	32.5	31.0
2	70 (0)	60 (+1)	10 (-1)	10.6	10.8
3	50 (-1)	20 (-1)	30 (0)	21.8	24.4
4	50 (-1)	60 (+1)	30 (0)	23.4	24.4
5	90 (+1)	40 (0)	50 (+1)	31.5	32.7
6	70 (0)	40 (0)	30 (0)	32.3	31.0
7	90 (+1)	60 (+1)	30 (0)	30.2	27.1
8	50 (-1)	40 (0)	10 (-1)	13.8	12.1
9	70 (0)	40 (0)	30 (0)	28	31.0
10	70 (0)	40 (0)	30 (0)	32.6	31.0
11	70 (0)	60 (-1)	10 (-1)	19.5	10.8
12	90 (+1)	40 (0)	10 (-1)	13.4	15.4
13	50 (-1)	40 (0)	50 (+1)	28	25.6
14	70 (0)	40 (0)	30 (0)	30.7	31.0
15	70 (0)	20 (-1)	50 (+1)	29.4	28.7
16	70 (0)	60 (+1)	50 (+1)	30.2	31.1
17	90 (+1)	20 (-1)	30 (0)	33.7	32.2

MTT assay as described with some modification. Briefly, SGC-7901 was seeded at a density of 2×10^4 cells per well in 96-well plate, grown for 24 h, and then treated with different concentration of green tea extracts for 24 h. After incubation, 20 µL MTT (5 mg/mL in PBS) was added to each well and the cells were incubated at 37 °C for 4 h. The supernatants were discarded and 150 µL DMSO was added to each well to dissolve the precipitate and the absorbance of the well was measured at 570 nm with a microplate reader (Thermo Scientific Multiskan GO, Vantaa, Finland). Results were expressed as percentage of cell viability (%), assuming control cells as 100%.

Statistical analysis

All experiments were performed in triplicate and centered. Analysis of variance (ANOVA) was performed by ANOVA procedure. $P < 0.05$ and $P < 0.01$ were regarded as significant and very significant, respectively. The optimal extraction conditions were established based on regression analysis and plotting of three-dimensional (3D) response surface plots.

Results and discussion

Single-factor experiment of EGCG extraction

Effect of temperature on the content of EGCG extraction

The effect of temperature on the EGCG extraction yield using an extraction time of 30 min, a solution pH of 6, water-tea ratio of 30 mL/g and extraction once, is shown in Fig. 1a. It demonstrates that the extraction temperature significantly affected the EGCG extraction yield. The EGCG yield increased significantly as the temperature increased from 50 to 70 °C. This could be explained by the fact that the rise in temperature can decrease solvent viscosity and surface tension, increase the solubility and diffusion rate of EGCG (Kong et al., 2010; Vuong et al., 2010; Vuong et al., 2011). However, beyond 70 °C, the EGCG yield decreased, suggesting that the higher extraction temperatures induced the concurrent decomposition of the compounds. This result was in agreement with several previous studies (Kong et al., 2010; Vuong et al., 2011). Therefore, a temperature of 70 °C was chosen as the central point for the RSM.

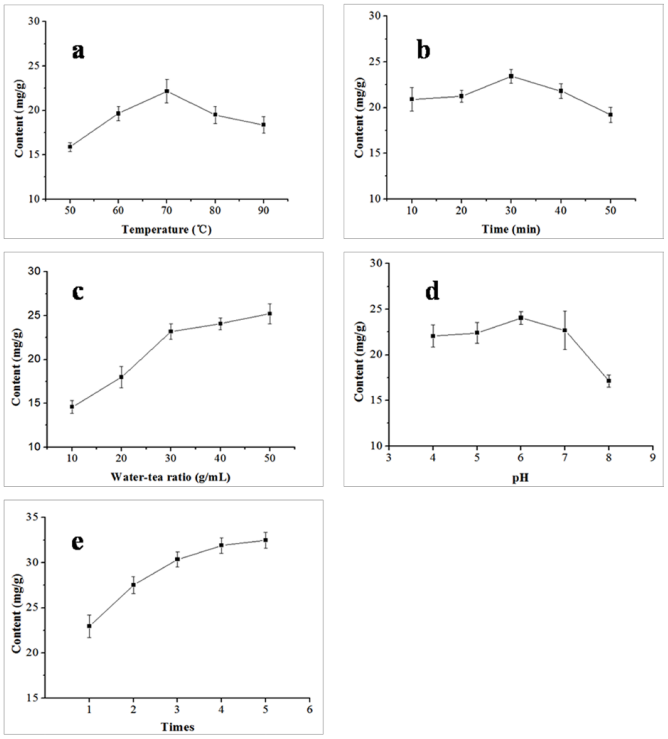

Fig. 1: Effect of extraction temperature (a), time (b), water-tea ratio (c), brewing solution pH (d), and extraction times (e) on the extraction yield of EGCG

Effect of time on the content of EGCG extraction

The EGCG was extracted at extraction time varying from 10 to 50 min and a temperature of 70 °C, a solution pH of 6, water-tea ratio of 30 mL/g and extraction once. As shown in Fig. 1b, a positive effect was caused by a longer extraction time. When extraction time changed from 10 min to 30 min, the EGCG yield increased from 20.91 ± 1.30 to 23.42 ± 0.74 mg/g due to the increase in reactive sites (Chen et al., 2017). However, beyond 30 min, the EGCG yield decreased, suggesting that prolonging extraction time may expose the EGCG to oxidative degradation (Kong et al., 2010). Thus, an optimal extraction time of 30 min was chosen as the central point for the RSM.

Effect of water-tea ratio on the content of EGCG extraction

The effect of water-tea ratio on EGCG yield was investigated under fixed conditions (a temperature of 70 °C, an extraction time of 30 min, a solution pH of 6 and extraction once). Fig. 1c exhibits that the yield of EGCG significantly increased from 14.57 ± 0.71 to 23.17 ± 0.88 mg/g when the ratio rose from 10:1 to 30:1 mL/g because of the increasing contact surface area between tea powders and water, and the increase of the solid-liquid concentration gradient as well as the diffusion rate in the extraction (Yang et al., 2017). However, further increase in the ratio did not significantly improve the extraction yield ($p > 0.05$), which is in agreement with several previous studies (Felkai-Haddache et al., 2016). Thus, a water-tea ratio of 30:1 mL/g was selected as the central point for the RSM.

Effect of pH on the content of EGCG extraction

The EGCG was extracted at solution pH varying from 4 to 8 and a time of 30 min, a temperature of 70 °C, water-tea ratio of 30 mL/g and extraction once. As shown in Fig. 1d, pH level showed a positive effect on the EGCG extraction ratio, which reached at 24.04 ± 0.71 mg/g at pH = 6 and then decreased rapidly. This is due to the fact that EGCG was quite stable in acidic solutions but its stability decreased when the pH of the extraction solution rose from pH 4 to 8 (Vuong et al., 2013). Consequently, a solution pH of 6 was selected as the central point for the RSM.

Effect of extraction times on the content of EGCG extraction

The effect of the extraction times on EGCG yield was investigated under fixed conditions (a temperature of 70 °C, an extraction time of 30 min, a solution pH of 6 and water-tea ratio of 30 mL/g). Fig. 1e showed that the extraction ratio increased significantly with the increasing number of extraction times at first. However, the extraction ratio increased slightly with the increasing number of extraction times after extraction twice. This could be explained by the fact that most of EGCG had been dissolved out in the first two extraction cycle. And this tendency was also in accordance with previous studies (Yang et al., 2017). Thus, the number of extraction times was fixed as twice in the RSM.

Optimization of EGCG extraction by response surface designs

According to the above results of single factor experiment, the three major parameters (extraction temperature, time and water-tea ratio) and their ranges for EGCG extraction were determined and adopted for Box-Behnken design with response surface methodology with the consideration of cost, energy and solvent consumption.

Model fitting

Tab. 3 showed the design matrix and the experimental and predicted values of the response. According to the multiple regression analysis, the fitted second-order polynomial model for EGCG in coded variables was shown in the following Eq. (2).

$$Y = -35.2775 + 1.0123X_1 + 0.2983X_2 + 1.0528X_3 - 0.0032X_1X_2 + 0.0024X_1X_3 + 0.0061X_2X_3 - 0.0059X_1^2 - 0.004X_2^2 - 0.018X_3^2 \quad (2)$$

where Y represents the EGCG extraction ratio, X_1, X_2 and X_3 represent the experimental value of extraction temperature, time, and water-tea ratio, respectively.

In order to determine whether the quadratic model is significant, it is necessary to run ANOVA analysis. The significance of each coefficient was measured using F-values and p-values. The smaller the p-value and the greater the F-value, the more significant the corresponding coefficient (Gan and Latiff, 2011; Hu et al., 2016). The regression coefficients of the linear, quadratic and interactive parameters are shown in Tab. 4. Lack of fit was also given in Tab. 4 in order to confirm model fit. The p-value of the model was less than 0.001, which indicated that the fitting model is adequate to describe the experimental data. F-value for the lack of fit was insignificant ($p > 0.05$) imply that the model is valid.

Among all variables, the linear of X_1 and X_3, and the quadratic of X_3^2 exhibited significant effects ($p < 0.05$), whereas the others had negligible effects on the EGCG extraction ratio ($p > 0.05$). The linear variable of water-tea ratio had the highest influence on the EGCG content to its p-value below 0.001, followed by the linear effect of extraction temperature. However, extraction time had no remarkable effect ($p > 0.05$). Besides, the quadratic effect of water-tea ratio has the significant influence on the content of EGCG ($p < 0.001$). It could be concluded that water-tea ratio played the most important role in the EGCG extraction among three independent variables, followed by extraction temperature and extraction time.

Tab. 4: ANOVA of the regression model for the prediction of MLP extractions.

Source	Sum of squares	DF	Mean square	F value	p-value Prob > F
Model	851.82	9	94.65	12.85	$p < 0.001$
X_1	59.41	1	59.41	8.07	$p < 0.05$
X_2	12.5	1	12.5	1.7	$p > 0.05$
X_3	477.4	1	477.4	64.82	$p < 0.001$
X_1X_2	6.5	1	6.5	0.88	$p > 0.05$
X_1X_3	3.8	1	3.8	0.52	$p > 0.05$
X_2X_3	23.52	1	23.52	3.19	$p > 0.05$
X_1^2	23.2	1	23.2	3.15	$p > 0.05$
X_2^2	10.75	1	10.75	1.46	$p > 0.05$
X_3^2	218.12	1	218.12	29.62	$p < 0.001$
Residual	51.55	7	7.36	-	-
Lack of fit	36.21	3	12.07	3.15	$p > 0.05$
Pure error	15.35	4	3.84	-	-
Total	903.37	16	-	-	-

Analysis of response surface plot and contour plot

The three-dimensional response surface plots and two-dimensional contour plots were applied to visualize the interactive effects of the independent variables on the EGCG yield (Fig. 2). The surface response plots of the model were created by changing two variables within their test range and keeping the other variable at their central level, which is the best way of showing the effect of the independent variable on the content of EGCG (Felkai-Haddache et al., 2016; Guo et al., 2016). The 3D response surface and 2D contour plots are the graphical representations of regression equation. Through these plots, it is easy to judge interactions between two variables and deter-

mine optimum ranges for independent variables (DAHMOUNE et al., 2014; ZHANG et al., 2011).

The variables of temperature/time, temperature/water-tea ratio, and time/water-tea ratio collaboratively worked in a nonlinear synergistic manner to the content of EGCG (Fig. 2A, 2B, and 2C). As it can be found from Fig. 2A and Fig. 2C, the response curve of extraction time was smooth. It indicated that the extraction time is less significant than other two effects. In Fig. 2B and Fig. 2C, water-tea ratio has the huge influence on the content of EGCG. With the increase of water-tea ratio from 30 to 41 mL/g, the content of EGCG increased to a definite value and subsequently maintained stable or decrease. The content of EGCG was not found to be significantly affected by an enhancement of the extraction temperature at a fixed water-tea ratio and extraction time, shown in Fig. 2A and Fig. 2B. This suggested that the change in extraction temperature had an alternative influence on the content of EGCG.

Model verification

By applying the regression analysis to Eqs. (1) and (2), the predicted optimized conditions were concluded as follows: X_1= 85.42 °C, X_2 = 34.22 min, and X_3 = 40.79 mL/g. Based on Eq. (2), the predicted maximum extraction yield of EGCG was 34.54 mg/g. For convenient operation in actual experiments, the verification experiment was performed under predicted optimal conditions with slightly modification as follows: temperature of 85 °C, time of 34 min, water-tea ratio of 41 mL/g, a solution pH of 6 and extraction twice. Under these conditions, the experimental extraction yield value of EGCG was 33.82 mg/g, which was no significant difference to predicted value. Therefore, the results revealed the accuracy and adequacy of the response model in the optimization of extraction process of EGCG from the lower grade green tea (LIM et al., 2017; YANG et al., 2017).

Effect of brewing conditions on the content of individual catechins

The concentrations of eight main tea catechins in green tea extract made with two brewing methods were quantified by HPLC (Tab. 5). The HPLC profile is shown in Supplementary materials 1. Compared with the TTBM group, the contents of all kinds of tested catechins in ITBM group were much higher, especially for the contents of C, CG, GCG, EGCG. Additionally, the contents of gallated catechins and total catechins in ITBM group were significantly higher than TTBM group ($p < 0.001$ and $p < 0.05$, respectively).

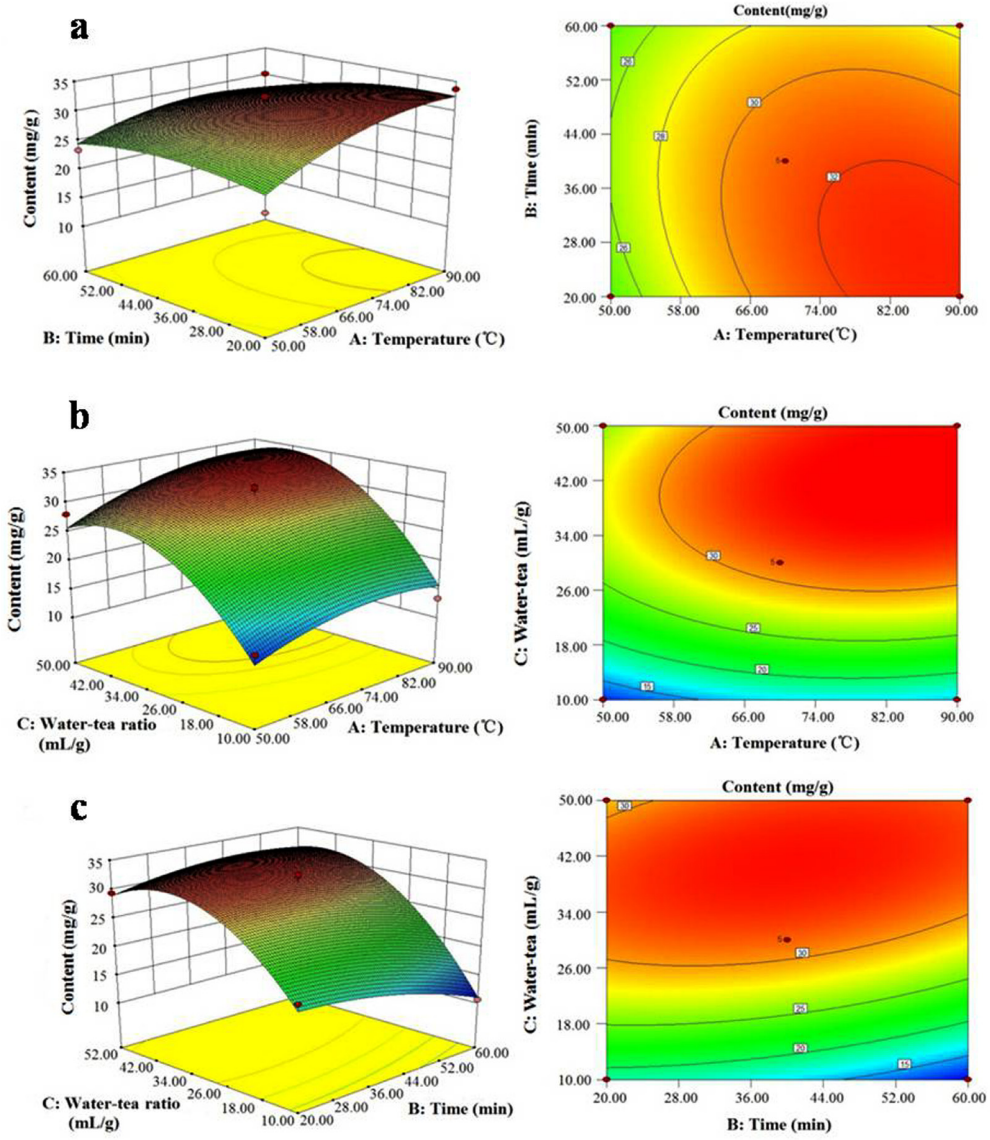

Fig. 2: Three-dimensional response surfaces and contour plots for the effect of variables on the extraction yield of EGCG

Tab. 5: Catechin contents of green tea extract brewing with TTBM and ITBM (mg/L).

Catechins	TTBM	ITBM
GC	163.34±0.54	164.43±1.69
EGC	256.06±6.05	256.31±4.81
C	28.58±0.83	36.11±0.76**
EC	68.8±5.28	72.5±2.15
CG	4.15±0.67	13.68±0.79**
ECG	1.59±0.16	3.14±0.12
GCG	41.93±3.46	52.66±2.87*
EGCG	281.98±16.80	340.07±13.77*
Gallated catechins	329.65±21.56	409.55±17.1**
Total catechins	846.43±25.63	938.92±25.95*

TTBM, traditional tea brewing method.
ITBM, improved tea brewing method.
Values are presented as means ± SD (n = 3).
* $p < 0.05$, ** $p < 0.01$ versus TTBM group.

Antitumor activity

Gastric cancer is one of the most serious malignant tumors, which is the leading cause of cancer-related death in most countries. Case-control studies have suggested that green tea consumption has protective effect of against gastric cancer (Xu et al., 2010; Zhu et al., 2007). It is reported that EGCG suppresses tumor growth by inhibiting proliferation and inducing apoptosis through a variety of mechanisms. In this study, we investigated cell growth inhibition effect of above green tea extract made with two different brewing methods on human gastric cancer SGC-7901 cells. Cells were treated with 1.5, 2.5 and 3.5 mg/mL of green tea extract for 24 h and cell viability was determined using the MTT assay. As shown in Fig. 3, green tea extract had significant growth inhibition effect on gastric cancer cells in a dose-dependent manner with a maximum loss at concentration of 3.5 mg/mL. Moreover, the decrease of the cell viability exposed to green tea extract made with ITBM was more significant than that of TTBM, which was consistent with the results of HPLC analysis. This indicated that the antitumor activity of green tea extract was due to the cumulative effects of tea catechins (Cooper et al., 2005).

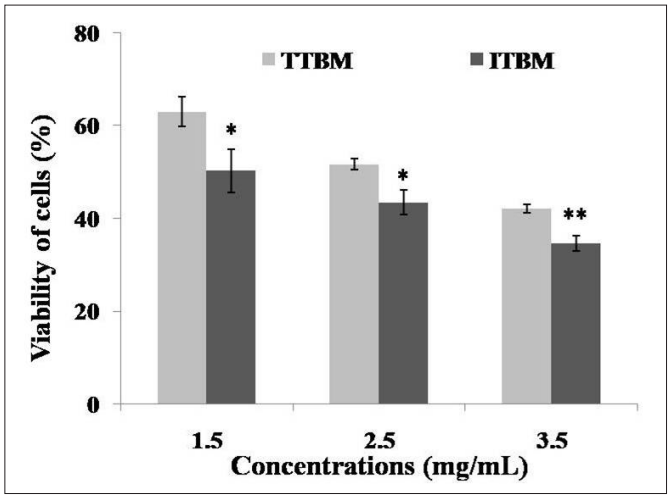

Fig. 3: Antitumor activities of green tea extract brewing with TTBM and ITBM against human gastric cancer SGC-7901 cells.
The cells were exposed tea extract of different concentrations (1.5, 2.5, 3.5 mg/mL) for 24 h respectively, and the cell viability was measured by the MTT assay as showed in materials and methods. The values were represented as the percent viable cells, where untreated cells were regarded as 100% viable. Data were mean ± SD, n = 5 (* $p < 0.05$; ** $p < 0.01$).

Conclusions

In the present study, Box-Behnken design based on the response surface methodology was successfully employed to optimize the EGCG extraction from summer green tea. The optimal extraction conditions for EGCG were: temperature of 85 °C, time of 34 min, water-tea ratio of 41 mL/g, a solution pH of 6, and extraction twice. Water-tea ratio was also found to have the major influence on the yield of EGCG. Under these conditions, the experimental extraction yield value of EGCG was 33.82 mg/g. Furthermore, the summer green tea extract prepared under the optimal conditions had the higher antitumor activity against human gastric cancer SGC-7901 cells. The results indicated that the rich and valuable resource of summer green tea can be utilized much better by using the optimal extraction conditions (Zhang et al., 2012). It is worth mentioning, however, that from the point of view of industrial application, future work need to be done to establish the pilot-plant trials of EGCG extraction and to claim the mechanism of the healthy benefits of summer green tea benifits in human body or food industry (Dahmoune et al., 2014).

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References

Castiglioni, S., Damiani, E., Astolfi, P., Carloni, P., 2015: Influence of steeping conditions (time, temperature, and particle size) on antioxidant properties and sensory attributes of some white and green teas. Int. J. Food Sci. Nutr. 66, 491-497. DOI: 10.3109/09637486.2015.1042842

Chen, C., Zhang, B., Huang, Q., Fu, X., Liu, R.H., 2017: Microwave-assisted extraction of polysaccharides from *Moringa oleifera* Lam. leaves: Characterization and hypoglycemic activity. Ind. Crop. Prod. 100, 1-11. DOI: 10.1016/j.indcrop.2017.01.042

Chen, Y.K., Cheung, C., Reuhl, K.R., Liu, A.B., Lee, M.J., Lu, Y.P., Yang, C.S., 2011: Effects of green tea polyphenol (–)-epigallocatechin-3-gallate on newly developed high-fat/western-style diet-induced obesity and metabolic syndrome in mice. J. Agric. Food Chem. 59, 11862-11871. DOI: 10.1021/jf2029016

Cooper, R., Morre, J., Morre, D.M., 2005: Medicinal benefits of green tea: Part II. Review of anticancer properties. J. Altern. Complement. Med. 11, 639-652. DOI: 10.1089/acm.2005.11.639

D'Archivio, A.A., Maggi, M.A., Ruggieri, F., 2016: Investigation by response surface methodology of extraction of caffeine, gallic acid and selected catechins from tea using water-ethanol mixtures. Food Anal. Methods 9, 2773-2779. DOI: 10.1007/s12161-016-0469-7

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., Madani, K., 2014: *Pistacia lentiscus* leaves as a source of phenolic compounds:

Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. Ind. Crop. Prod. 61, 31-40. DOI: 10.1016/j.indcrop.2014.06.035

Dalar, A., Konczak, I., 2013: Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. Ind. Crop. Prod. 44, 383-390. DOI: 10.1016/j.indcrop.2012.11.037

Felkai-Haddache, L., Dahmoune, F., Remini, H., Lefsih, K., Mouni, L., Madani, K., 2016: Microwave optimization of mucilage extraction from *Opuntia ficus indica* Cladodes. Int. J. Biol. Macromol. 84, 24-30. DOI: 10.1016/j.ijbiomac.2015.11.090

Gadkari, P.V., Balarman, M., Kadimi, U.S., 2015: Polyphenols from fresh frozen tea leaves (*Camellia assamica* L.) by supercritical carbon dioxide extraction with ethanol entrainer - application of response surface methodology. J. Food Sci. Tech. Mys. 52, 720-730. DOI: 10.1007/s13197-013-1085-9

Gan, C.Y., Latiff, A.A., 2011: Optimisation of the solvent extraction of bioactive compounds from Parkia speciosa pod using response surface methodology. Food Chem. 124, 1277-1283. DOI: 10.1016/j.foodchem.2010.07.074

Garbisa, S., Sartor, L., Biggin, S., Salvato, B., Benelli, R., Albini, A., 2001: Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer 91, 822-832. DOI: 10.1002/1097-0142(20010215)91:4<822::aid-cncr1070>3.0.co;2-g

Guo, L., Guo, J., Zhu, W., Jiang, X., 2016: Optimized synchronous extraction process of tea polyphenols and polysaccharides from Huaguoshan Yunwu tea and their antioxidant activities. Food Bioprod. Process. 100, 303-310. DOI: 10.1016/j.fbp.2016.08.001

Hajiaghaalipour, F., Sanusi, J., Kanthimathi, M.S., 2016: Temperature and time of steeping affect the antioxidant properties of white, green, and black tea infusions. J. Food Sci. 81, 246-254. DOI: 10.1111/1750-3841.13149

Hininger-Favier, I., Benaraba, R., Coves, S., Anderson, R.A., Roussel, A.-M., 2009: Green tea extract decreases oxidative stress and improves insulin sensitivity in an animal model of insulin resistance, the fructose-fed rat. J. Am. Coll. Nutr. 28, 355-361. DOI: 10.1080/07315724.2009.10718097

Hu, C., Gu, C., Fang, Q., Wang, Q., Xia, Q., 2016: Transdermal solid delivery of epigallocatechin-3-gallate using self-double-emulsifying drug delivery system as vehicle: Formulation, evaluation and vesicle-skin interaction. J. Biomater. Appl. 30, 1080-1091. DOI: 10.1177/0885328215617891

Kao, Y.H., Chang, H.H., Lee, M.J., Chen, C.L., 2006: Tea, obesity, and diabetes. Mol. Nutr. Food Res. 50, 188-210. DOI: 10.1002/mnfr.200500109

Khan, N., Mukhtar, H., 2007: Tea polyphenols for health promotion. Life Sci. 81, 519-533. DOI: 10.1016/j.lfs.2007.06.011

Kong, K.W., Ismail, A.R., Tan, S.T., Prasad, N., Murthy, K., Ismail, A., 2010: Response surface optimisation for the extraction of phenolics and flavonoids from a pink guava puree industrial by-product. Int. J. Food Sci. Tech. 45, 1739-1745. DOI: 10.1111/j.1365-2621.2010.02335.x

Lim, C.J., Basri, M., Ee, G.C.L., Omar, D., 2017: Phytoinhibitory activities and extraction optimization of potent invasive plants as eco-friendly weed suppressant against *Echinochloa colona* (L.) Link. Ind. Crop. Prod. 100, 19-34. DOI: 10.1016/j.indcrop.2017.01.025

Liu, Y., Bai, Q., Lou, S., Di, D., Li, J., Guo, M., 2012: Adsorption characteristics of (–)-epigallocatechin gallate and caffeine in the extract of waste tea on macroporous adsorption resins functionalized with chloromethyl, amino, and phenylamino groups. J. Agric. Food Chem. 60, 1555-1566. DOI: 10.1021/jf204710h

Pavlovic, M.D., Buntic, A.V., Siler-Marinkovic, S.S., Antonovic, D.G., Dimitrijevic-Brankovic, S.I., 2015: Recovery of (–)-epigallocatechingallate (EGCG) from aqueous solution by selective adsorption onto spent coffee grounds. Eur. Food Res. Technol. 241, 399-412. DOI: 10.1007/s00217-015-2472-4

Perva-Uzunalić, A., Škerget, M., Knez, Ž., Weinreich, B., Otto, F., Grüner, S., 2006: Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. Food Chem. 96, 597-605. DOI: 10.1016/j.foodchem.2005.03.015

Sae-tan, S., Grove, K.A., Lambert, J.D., 2011: Weight control and prevention of metabolic syndrome by green tea. Pharmacol. Res. 64, 146-154. DOI: 10.1016/j.phrs.2010.12.013

Saklar, S., Ertas, E., Ozdemir, I.S., Karadeniz, B., 2015: Effects of different brewing conditions on catechin content and sensory acceptance in Turkish green tea infusions. J. Food Sci. Tech. Mys. 52, 6639-6646. DOI: 10.1007/s13197-015-1746-y

Savic, I., Nikolic, V., Savic-Gajic, I., Nikolic, L.B., Moder, K., Hopkins, M., 2016: Optimization of quercetin extraction from green tea (*Camellia sinensis*) using central composite design, and the pharmacological activity of the extract. Chem. Biochem. Eng. Q. 30, 103-115. DOI: 10.15255/CABEQ.2015.2166

Savic, I.M., Nikolic, V.D., Savic-Gajic, I.M., Nikolic, L.B., Ibric, S.R., Gajic, D.G., 2015: Optimization of technological procedure for amygdalin isolation from plum seeds (*Pruni domesticae* semen). Front. Plant Sci. 6. DOI: 10.3389/fpls.2015.00276

Savić, I.M., Nikolić, V.D., Savić, I.M., Nikolić, L.B., Stanković, M.Z., Moder, K., 2013: Optimization of total flavonoid compound extraction from *Camellia sinesis* using the artificial neural network and response surface methodology. Hem. Ind. 67, 249-259. DOI: 10.2298/HEMIND120313066S

Singh, B.N., Shankar, S., Srivastava, R.K., 2011: Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and clinical applications. Biochem. Pharmacol. 82, 1807-1821. DOI: 10.1016/j.bcp.2011.07.093

Vuong, Q.V., Golding, J.B., Nguyen, M., Roach, P.D., 2010: Extraction and isolation of catechins from tea. J. Sep. Sci. 33, 3415-3428. DOI: 10.1002/jssc.201000438

Vuong, Q.V., Golding, J.B., Stathopoulos, C.E., Nguyen, M.H., Roach, P.D., 2011: Optimizing conditions for the extraction of catechins from green tea using hot water. J. Sep. Sci. 34, 3099-3106. DOI: 10.1002/jssc.201000863

Vuong, Q.V., Golding, J.B., Stathopoulos, C.E., Roach, P.D., 2013: Effects of aqueous brewing solution pH on the extraction of the major green tea constituents. Food Res. Int. 53, 713-719. DOI: 10.1016/j.foodres.2012.09.017

Wheeler, D.S., Wheeler, W.J., 2004: The medicinal chemistry of tea. Drug Dev. Res. 61, 45-65. DOI: 10.1002/ddr.10341

Xu, X.X., Jiang, H.R., Li, H.B., Zhang, T.N., Zhou, Q., Liu, N., 2010: Apoptosis of stomach cancer cell SGC-7901 and regulation of Akt signaling way induced by bovine lactoferrin. J. Dairy Sci. 93, 2344-2350. DOI: 10.3168/jds.2009-2926

Yang, S., Li, Y., Jia, D., Yao, K., Liu, W., 2017: The synergy of Box-Behnken designs on the optimization of polysaccharide extraction from mulberry leaves. Ind. Crop. Prod. 99, 70-78. DOI: 10.1016/j.indcrop.2017.01.024

Zhang, G., He, L., Hu, M., 2011: Optimized ultrasonic-assisted extraction of flavonoids from *Prunella vulgaris* L. and evaluation of antioxidant activities *in vitro*. Innov. Food Sci. Emerg. 12, 18-25. DOI: 10.1016/j.ifset.2010.12.003

Zhang, X., Xu, F., Gao, Y., Wu, J., Sun, Y., Zeng, X., 2012: Optimising the extraction of tea polyphenols, (-)-epigallocatechin gallate and theanine from summer green tea by using response surface methodo-logy. Int. J. Food Sci. Technol. 47, 2151-2157. DOI: 10.1111/j.1365-2621.2012.03082.x

Zhao, Z.Y., Huangfu, L.T., Dong, L.L., Liu, S.L., 2014: Functional groups and antioxidant activities of polysaccharides from five categories of tea. Ind. Crop. Prod. 58, 31-35. DOI: 10.1016/j.indcrop.2014.04.004

Zhu, B.-H., Zhan, W.H., Li, Z.R., Wang, Z., He, Y.L., Peng, J.S., Cai, S.R., Ma, J.P., Zhang, C.H., 2007: (-)-Epigallocatechin-3-gallate inhibits growth of gastric cancer by reducing VEGF production and angiogenesis. World J. Gastroentero. 13, 1162-1169. DOI: 10.3748/wjg.v13.i8.1162

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