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Optimization Research into the Ultrasonic-assisted Extraction to Separate Polyphenol from Green Tea Waste

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Today, green tea leaves (*Camellia sinensis*) have been grown in nearly 30 provinces in Vietnam. Catechins, which are polyphenols, are abundant in green tea. In a freshly picked tea leaf, polyphenols can compose up to 30% of the dry weight. Recently, researchs have focused on tea polyphenols due to its potent anti-oxidant activity, anti-inflammatory and anti-cancer effects. Viet Nam currently ranks the fifth in the world in tea production and export turn-over. However, green tea is made from the top two leaves and buds of a shrub. A large amount of stems, older leaves and tea wastes from tea factories is not produced. For this reason, study to separation polyphenols from these materials plays an important role in science, society and economy.

Polyphenols can be extracted from green tea leaves by using hot water and organic solvents. In the method of using hot water, green tea quality deteriorates and catechins are destroyed due to the heat applied. Therefore, we attempted to increase the amount of polyphenols in extracts with using ultrasonic irradiation at low temperature.

This work has an aim to investigate the influent process parameters such as extraction time, stirring speed and the rate of raw material/solvent on efficiency of the extraction of polyphenols and antioxidant properties obtained extracts. The ultrasonic-assisted extraction was conducted at room temperature, ultrasound frequency (25 kHz) using water solvent. Extracts were analysed for total phenols content (TPC) by the Lowenthal method. The antioxidant properties have been determined by DPPH free radical scavenging effect, The results of the work are the basis to determine of the optimal technological factors by employing desirability methodology with experimental order of

Box-Behnken design and to scale up for designed industrial extraction system that can be applied in tea plants for the production of tea polyphenols from tea wastes.

Keywords : Ultrasonic, Extraction, Polyphenol, Green tea waste, Optimization

INTRODUCTION

Green tea has been one of popular drinks in Asian countries and now is widely used in all over the world due to its benefits for human health. Green tea is rich in flavonoids and polyphenols that have been shown to possess a wide range of biological and pharmaceutical effects, including antioxidation, anticancer, and anti-inflammation (Gupta *et al*., 2002; Kuroda and Hara, 1999; Moyers and Kumar, 2004). These polyphenols, tea main components, which may be as high as 30 – 35 % in dried weight of fresh tea leaves.

In Vietnam, tea leaves have been grown in nearly 30 provinces and Viet Nam currently ranks the fifth in the world in tea production and export turn-over. However, green tea is made from the top two leaves and buds of a shrub. A large amount of stems, older leaves and tea wastes from tea factories is not produced. Therefore research into separation polyphenols from these materials plays an important role in science, society and economy.

The extraction of polyphenols is difficult for two main reasons:

- First, these compounds are extremely different in relation to their structure – they may occur in plant tissues combined with sugars, proteins or they may create polymerized derivatives of various dissolubility. Their chemical structure and interactions with other food components are not fully known and it is a very important information at the selection of a solvent and terms and conditions of extraction.
- Second, polyphenols are susceptible to oxidation, furthermore high temperature and alkaline environment cause their degradation, thus the extraction process and further stages of the preparation of a trial to specify the contents of polyphenols are subject of great caution (Tura and Robards 2002).

In previous researches (Phung *et al.* , 2009, Gu *et al*., 2007), ultrasonic assisted-extraction using ethanol solvent for separation polyphenols green tea has been reported. In this research, the efficiency of the process can be regulated by the selection of water solvent and application stirring.

This paper, therefore, focuses on the influent factors like extraction time, stirring speed, the rate of raw material/solvent on efficiency of the extraction of polyphenols from green tea waste and antioxidant properties obtained extracts and determination of the optimization parameters. Desirability methodology and Box-Behnken design were employed to optimize polyphenol extraction from aged tea leaves by water solvent.

EXPERIMENTAL

Material

The research material was tea waste product from Tea Corporation Hiep Khanh. These green tea leaves are harvested in the main tea areas in the North of Vietnam: Hoa Binh, Son La, Ha Giang, Tuyen Quang , etc.

There are a lot of tea waste products but in this work, we choose the BMF sample, that includes small crude and waste tea leaves with moisture <8%.

Fig.1 : Picture of sample (BMF)

Tea extracts preparation: approximately 3g ground and dried tea leaves was weighed, then it was placed in a 250ml flask with a condenser. The extraction was carried out in ultrasonic bath at defined condition of time, temperature, solvent/material ratio and stirring speede. The crude extract was then filtrated, vacuum concentrated and adjusted to 100ml by distilled water. The tea extracts were kept in tight bottle and stored in refrigerator.

Analysis methods

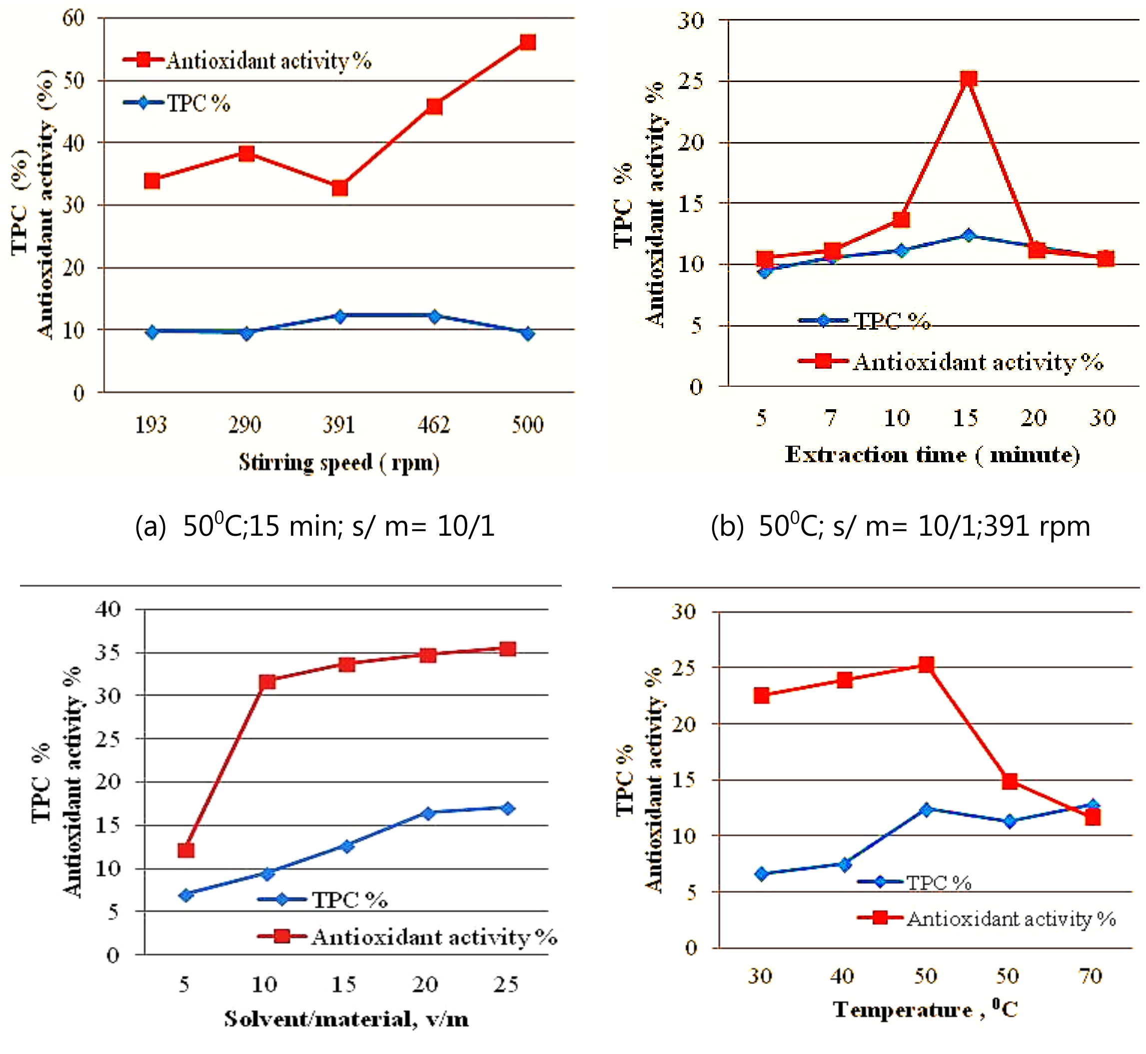
- Quantitative analysis of the total polyphenol content (TPC) in tea extracts was carried out using Lowenthal method (AOAC, 2000).
- Antioxydant activity of polyphenol was carried out by using the scavenging effect on DPPH radicals (Zhu Q.Y., 2002). All tea extracts prepared had the polyphenol content of 100 ppm.

Statistical analysis

Statistical experimental design demonstrates more satifactory and effective than other traditional methods which only allow to study one-at-a-time factor or mathematical methods because it can be used to study many variables simultaneously with less observations in saving time and costs. This method has been widely used in different research fields such as biology, food, agriculture and medicine. Desirability methodology is considered as an excellent method for multiple objective optimization of technical factors (Derringer G., 1980).

Statistical experimental design method: the Box-Behnken design for 3 factors: stirring speed, time, solvent/material ratio was used; each factor was carried out at 3 levels (see Table 1). This design has 17 trials including 12 trials for factorial design, 5 trials for central points with 2 responses as following: total phenols content (TPC)-Y_1 and antioxydant activity-Y_2 (see Table 1).

The statistical software Design-Expert 7.1 (Stat-Ease, Inc., Minneapolis, USA) was used for regression analysis of experimental data, to plot response surface and to optimize by desirability methodology. ANOVA was used to estimate the statistical parameters.



(a) 50^{0}C;15 min; s/ m= 10/1

(b) 50^{0}C; s/ m= 10/1;391 rpm

(c) 50^{0}C;15 min; 391 rpm

(d) 15 min; s/ m= 10/1; 391 rpm

Fig. 2: Dependence of TPC and antioxidant activity of extracts on a) stirring speed, b) extraction time, c) solvent/material ratio and d) temperature

RESULTS AND DISCUSSION

Study on the influence factors on polyphenol extraction

Figure 2 presents effect of the stirring speed, time, solvent/material ratio and temperature on the TPC and antioxidant of extracts. Based on the experimental results, the temperature has been fixed at 50^{0}C and the range of other influent factors have been defined for experimental design as follow:

- Stirring speed: 391 rpm to 460 rpm.
- Extraction time: 10 to 20 minutes.
- Solvent / material ratio: 15/1 to 25/1

Model building

The Box-Behnken design for 3 factors and results as shown in Table 1. The responses: TPC and antioxydant activity

Table 1. Experimental design and results

Run	Stirring speed (ram)	Time (min)	Solvent/material (v/m)	Code variables			Y_1	Y_2
				A	B	C		
1	390	10	20	-1	-1	0	13.26	16.83
2	460	10	20	1	-1	0	14.12	41.71
3	390	20	20	-1	1	0	13.30	27.20
4	460	20	20	1	1	0	13.27	48.99
5	390	15	15	-1	0	-1	11.60	24.37
6	460	15	15	1	0	-1	12.79	41.79
7	390	15	25	-1	0	1	16.20	29.37
8	460	15	25	1	0	1	16.45	58.54
9	425	10	15	0	-1	-1	12.87	33.16
10	425	20	15	0	1	-1	12.49	31.66
11	425	10	25	0	-1	1	16.73	35.18
12	425	20	25	0	1	1	16.49	54.89
13	425	15	20	0	0	0	15.36	52.51
14	425	15	20	0	0	0	15.65	52.74
15	425	15	20	0	0	0	15.36	54.65
16	425	15	20	0	0	0	14.84	56.80
17	425	15	20	0	0	0	14.98	57.99

Table 2a. Regression analysis of TPC (Y_1)

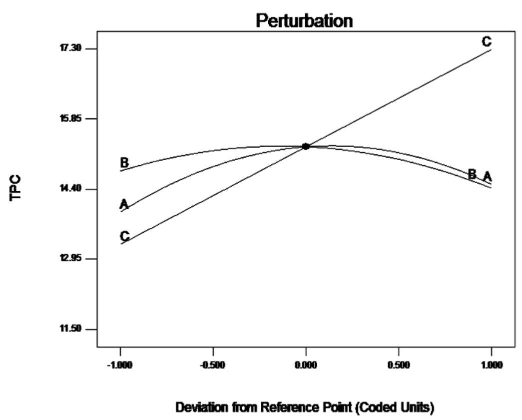
Source	Mean square	F-value	p value (prob>F)
Model	5.84	99.05	< 0.0001
A	0.65	10.96	0.0091
B	0.26	4.38	0.0659
C	32.47	551.08	<0.0001
AB	0.20	3.32	0.1016
AC	0.22	3.75	0.0848
A^2	4.76	80.81	<0.0001
B^2	1.94	32.93	0.0003
Lack of fit	0.02	0.21	0.9435

were fitted with a second-order polynomial equations of three factors.

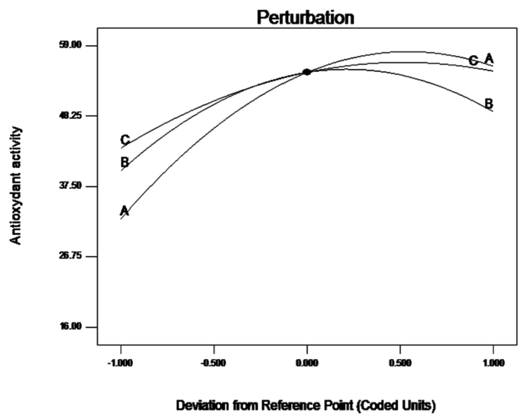
The significance of model and coefficients controlled by regression analysis (Table 2a, 2b). F-values of two models are 99.05 (Y_1), 104.26 (Y_2) in turn showed that all two models are completely significant of 99.99% ($p<0.0001$) confidence level. The p-value less than 0.05 indicates that the coefficient is significant, so in the Y_1 model coefficients A, C, A^2, B^2 were also significant, coefficients B, AB, AC were insignificant but they were remained un the model for optimization. Meanwhile, in Y_2 model only interactive coefficient AB was insignificant. F-value for "lack of fit" of

Table 2b. Regression analysis of Antioxydant activity (Y_2)

Source	Mean square	F-value	p value (prob>F)
Model	359.43	104.26	< 0.0001
A	1087.36	315.41	<0.0001
B	160.69	46.61	0.0001
C	276.03	80.07	<0.0001
AC	34.52	10.01	0.0133
BC	112.54	32.64	0.0004
A^2	485.03	140.69	<0.0001
B^2	466.51	135.32	<0.0001
C^2	136.29	39.53	0.0002
Lack of fit	0.99	0.17	0.9444



a



b

Fig. 3: Influence of factors on polyphenol extraction

Y_1 model and Y_2 model were 0.21 (p=0.9435) and 0.17 (p=0.9444), respectively. They showed that the two models were fit with experiment. In addition, the coefficients of determination (R^2) of the two models 0.9872 and 0.9905 in turn showed that the models were well matched with experiment designed.

The TPC and antioxydant actitvity of polyphenol are performed in second-degree model as followings:

$$Y_1 = 15.28 + 0.28A - 0.18B + 2.01C - 0.22AB - 0.24AC - 1.06A^2 - 0.68B^2 \quad (1)$$

$$Y_2 = 54.94 + 11.66A + 4.48B + 5.87C - 2.94AC + 5.30BC - 10.73A^2 - 10.53B^2 - 5.69C^2 \quad (2)$$

Considering in turn the effect of each factor (when other factors are remained in central level) on TPC (Figure 3a) and antioxydant activity of polyphenol (Figure 3b), it showed that the solvent/material

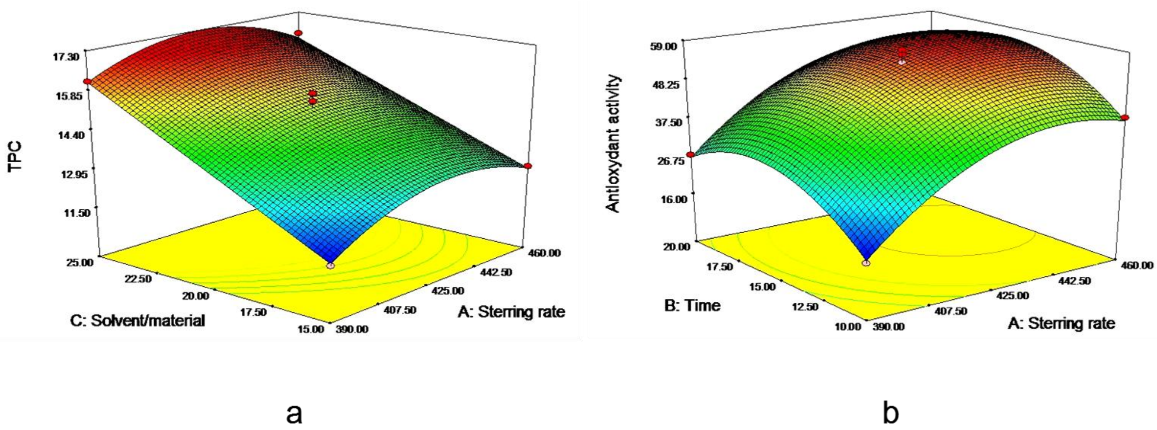


Fig. 4: Response surface plot for TPC and antioxydant activity of polyphenol

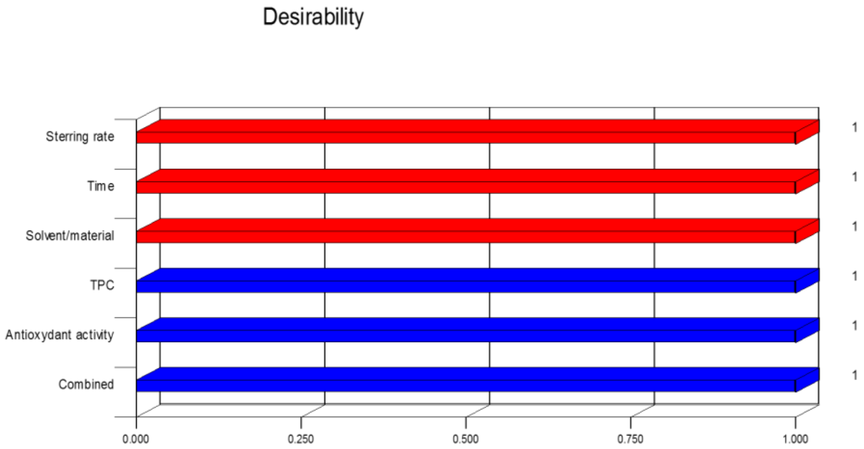


Fig. 5: Responsible desirability level

ratio (C) and sterring rate (A) have had a significant role on the TPC obtained; meanwhile sterring rate (A) and time (B) took an important role on antioxydant activity.

This can be shown clearly in the response surface of Y_1 function (Figure 4a) and Y_2 function (Figure 4b).

Optimization of the polyphenol extraction

The most important objective of polyphenol extraction process from green tea waste is to get the highest TPC and antioxydant activity. To this point, optimization by using algorism of fastened targets according to desirability methodology invented by Derringer and Suich was applied (Derringer G., 1980). The results after using Design-Expert 7.1 software were as followings: sterring rate 442 rpm, extraction time 16 minutes, solvent/material ratio 25/1 and extraction temperature 50°C, ultrasound frequency 25 kHz; in this condition, TPC and antioxydant activity of polyphenol have reached to 16.98% and 61.06%, respectively. The TPC, antioxydant activity and combined objective also have obtained 100%

desirability of objective proposed (Figure 5).

CONCLUSION

Ultrasonic – assisted extraction using water solvent and stirring for separation of polyphenols from green tea waste products is suitable, both in terms of technical and economic efficiency.

The influence of different conditions of ultrasonic extraction on the of polyphenols from green tea waste was studied. Various extraction times (10 to 20 min), and various stirring rates (391 to 460 rpm), various solvent/material ratios (15 to 25 v/m) and various temperatures (30 to 70^0C) were applied.

The results of this research show that the optimized process parameter are: stirring speed 442 rpm, extraction time 16 minutes, solvent/material ratio 25/1 and extraction temperature 50°C, ultrasound frequency 25 kHz. In this condition, TPC and antioxydant activity of polyphenol have reached to 16.98% and 61.06%, respectively.

On the basis of the results achieved large-scale production to separate polyphenols from green tea wastes will be further researched.

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