Southeast-Asian J. of Sciences Vol. 4, No. 1 (2015) pp. 19-29

Optimization of Ultrasonic Extraction of Polyphenols from Oolong Tea Byproducts Using Response Surface Methodology

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Abstract

Oolong tea, a popular drink with beneficial health properties, is a rich source of polyphenols that have a wealth of physiological activities. Ultrasound-assisted extraction (UAE) was applied for the extraction of total polyphenols from the byproducts of oolong tea. The predicted optimal conditions for the highest concentration of polyphenols were found at temperature of 55^0C, time of 45 minutes, ethanol 45% v/v, solid/liquid of 1:25, tea size ¡ 1 mm by UAE method (40 kHz, sonication power 250W). Antioxidant activity of three different materials of oolong tea was IC50 = 15.69; 19.07; 12.02 g/ml, respectively; IC50 of vitamin C was 16.47 g/ml.

1. Introduction

Tea is the most popular beverage in the world. It has been well-known for a long time that three kinds of tea, i.e., green tea, oolong tea, and black tea, have beneficial effects on health. All types of tea are manufactured from the same plant species, Camellia sinensis L., but the preparation process for each one is different: oolong tea is semi-fermented, green tea is unfermented, and black

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Key words: oolong tea, polyphenols, antioxidant, ultrasonic extraction, response surface methodology.

tea is well fermented. The polyphenols comprise one of the most distingishing characteristics of the tea plant and have been more thoroughly investigated than any other class of compounds in tea (25 - 30%) (Harbowy and Balentine, 1997). Tea polyphenols have been reported to have various biological, pharmacological functions and cosmetics for either enhancing product shelf-life or for enhancing human health (Hitoshi and Nobuyoshi, 2007). In these teas, aerobic oxidation of the tea leaf polyphenolics is allowed to occur and the catechins are enzymatically catalysed to form theaflavins and thearubigins.

Currently there are researches on extraction of polyphenols from tea in Vietnam (Vu Hong Son and Ha Duyen Tu, 2009; Pham Thanh Quan et al, 2005), but only on material research into the major tea green tea and the majority use traditional extraction methods. For oolong tea, which is the tea product line of high-end, high cost therefore the study take advantage of the byproducts such as shredded tea, tea bran during the production oolong tea for processing the product reasonable cost while maintaining quality as well as its characteristic flavor.

Polyphenols were extracted by different techniques such as traditional extraction using water solvent, the Soxhlet extraction, Microwave assisted extraction (MAE), Ultrasound-assisted extraction (UAE). The purpose of the present study was to develop a Ultrasound-assisted extraction (UAE) of the byproducts of oolong tea polyphenols. The application of ultrasound-assisted extraction (UAE) in food-processing technology is of interest for facilitating the extraction of components from plant materials. The higher yield achieved in these UAE processes is of major interest from an industrial standpoint, since the technology is an add-on step to the existing process with minimum alteration, application in aqueous extraction where organic solvents can be replaced with solvents generally recognised as safe (GRAS), reduction in solvent usage, and shorter extraction time. The use of ultrasonic means for extraction purposes in high-cost raw materials is an economical alternative to traditional extraction processes, this being a demand by industry for a sustainable development (Patricia et al, 2010).

2. Materials and Methods

2.1 Materials The byproducts of oolong tea (bran oolong tea) were purchased from Cautre Export Goods Processing Joint Stock Co. (Lamdong, Vietnam) and were pulverized to mean particle size of 1mm by a high speed blender, and stored at 4C for later use. Folin-Ciocalteu's phenol reagent, gallic acid, ascorbic acid, and 2,2-diphenyl-1-picrylhydrazil (DPPH) were purchased from Sigma-Aldrich Co. All the reagents used in the HPLC analyses were of HPLC grade.

2.2 Methods

Ultrasonic-assisted extraction The ultrasonic extraction was treated with an ultrasonic cleaning bath (WiseClean, Korea) with an ultrasonic power of 250 watts and a frequency of 40 kHz. A 10 g tea sample was mixed with 250 ml of distilled water in a 500 ml plastic flask that was placed in the water in the ultrasonic cleaning bath (Xia et al., 2006). The range values of the three independent variables were determined by preliminary study. The parameters of ultrasonic-assisted extraction were determined as follows: ultrasonic input power 250 W, extraction temperature 40, 50, 60oC; extraction time 30, 40, 50 min and ethanol 45, 50, 55% (v/v), on the basis of the analysis of results of the main components of extracted tea infusions.

Total polyphenols determination Total polyphenols were determined on the extract samples according to the Folin-Ciocalteu method (ISO 145021:2005), using gallic acid as the calibrant.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined according to the method of Blois with a slight modification (Qin et al., 2002). Briefly, a 0.1 mM solution of DPPH radical solution in ethanol was prepared, and then 2 ml of this solution was mixed with 300 l of extract solution in ethanol containing 10 - 50 g/ml of dried extract; the mixture was then vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. This activity is given as percent DPPH scavenging and is calculated as

%DPPH scavenging = [(control absorbance - extract absorbance) 100/control absorbance] (1)

Vitamin C was used as a control.

Effect of ultrasound

At all studied conditions, a significant positive effect of the ultrasound irradiation was observed. The effect of ultrasounds was calculated using following equation (Hitoshi and Nobuyoshi, 2007; d'Alessandro et al., 2012):

$$R(\%) = (B - A) \times 100/A \tag{2}$$

Where R is effect of ultrasound (%), B is the yield of polyphenols extracted with ultrasonic irradiation (%) and A is the yield of polyphenols extracted without irradiation (%).

Experimental Design and Statistical Analysis

Ultrasonic extraction optimized the experimental design using Response Surface Methodology RSM. A 15-run Box-Behnken design with three factors and three levels, including three replicates at the centre point, was used for fitting a second-order response surface. The independent variables were the extraction temperature (X1), extraction time (X2), and ethanol concentration (X3) while dependent variable (response) was total polyphenols contents (Y). The range values of the three independent variables were determined by preliminary study. Experiments were performed in three replicates and the average values were used as the response, Y. A full polynomial model was obtained with a multiple regression technique for three factors using JMP 9.0.2 (SAS Inc., USA).

Table 1. Box-Behnken design with experimental and predicted values of total polyphenols content

Run No.	Coded			Processed variables levels			Total polyphenols (%)	
	X_1	X_2	X_3	Extraction temperature (°C)	Extraction time (min)	Ethanol concentration (%)	Experimental	Predicted
1	-1	-1	0	40	30	50	11.59	11.623
2	-1	0	-1	40	40	45	12.35	12.333
3	-1	0	1	40	40	55	12.24	12.219
4	-1	1	0	40	50	50	12.36	12.377
5	0	-1	-1	50	30	45	12.56	12.557
6	0	-1	1	50	30	55	12.19	12.195
7	0	0	0	50	40	50	12.85	12.9
8	0	0	0	50	40	50	12.89	12.9
9	0	0	0	50	40	50	12.96	12.9
10	0	1	-1	50	50	45	13.11	13.115
11	0	1	1	50	50	55	12.91	12.917
12	1	-1	0	60	30	50	12.36	12.357
13	1	0	-1	60	40	45	13.08	13.119
14	1	0	1	60	40	55	12.64	12.673
15	1	1	0	60	50	50	12.91	12.883

3. Results and Discussion

Response Surface Optimization of Ultrasonic Extraction of Polyphenols from The Byproducts of Oolong Tea

For RSM, the levels of independent variables for the extraction of the polyphenols were selected based on the results obtained from our preliminary experiments. The experimental design and corresponding response data are presented in Table 1. The ranges of extraction temperature (40 - 60^{0}C), extraction time (30 - 50 min), and ethanol concentration (45% - 55%) were used. Total polyphenols were used as responses in the RSM experimental design. Predicted response Y for extraction of the polyphenols byproducts oolong tea extracts could be obtained by applying multiple regression analysis on the experimental data.

The fitness of the model was further confirmed by a satisfactory value of determination coefficient R2, which was calculated to be 0.991, indicating that 99.1% of the variability in the response could be predicted by the model. Lack of fit test for the model describes the variation in the data around the fitted model. If the model does not fit the data well, the value of lack of fit will be significant and then proceeding with investigation and optimization of the fitted response surface is likely to give misleading results. The models used to fit response variable were significant ($p < 0.001$) and the lack of fit was not significant ($p > 0.05$) for all responses (Table 3). It is indicated that the models used to fit responses variable were adequate to represent the relationship between the response values and the independent variables.

The regression coefficients, along with the corresponding P-values, for the model of polyphenol extraction from the byproducts of oolong tea were shown in Table 2. The P-values were used as a tool to check the significance of each coefficient, which also indicates the interaction effects between each independent variable. The regression of all the linear term and quadratic coefficients of X_1^2, X_2^2 and X_3^2. were significant and two cross-products (X_1X_2, X_1X_3 and X_2X_3) were also significant.

The polynomial model for total polyphenols content (Y) was regressed by considering only the significant terms and shown as below:

$Y = 12,9 + 0,31X_1 + 0,32X_2 - 0,14X_3 - 0,35X_1^2 - 0,24X_2^2 + 0,036X_3^2 - 0,057X_1X_2 - 0,083X_1X_3 + 0,041X_2X_3$

Table 2. Regression analysis of a full second-order polynomial model for optimisation of polyphenol extraction from the byproducts of oolong tea

Variables	Coefficient based on coded value	P-value
Intercept	12.9	< 0.0001*
X_1	0.31	< 0.0001*
X_2	0.32	< 0.0001*
X_3	-0.14	< 0.0001*
$X_1 \times X_1$	-0.35	<0.0001*
$X_2 \times X_2$	-0.24	< 0.0001*
$X_3 \times X_3$	0.036	0.0109*
$X_1 \times X_2$	-0.057	<0.0001*
$X_1 \times X_3$	-0.083	< 0.0001*
$X_2 \times X_3$	0.041	0.0030*
R^2	0.991	
R^2_{Adj}	0.9885	

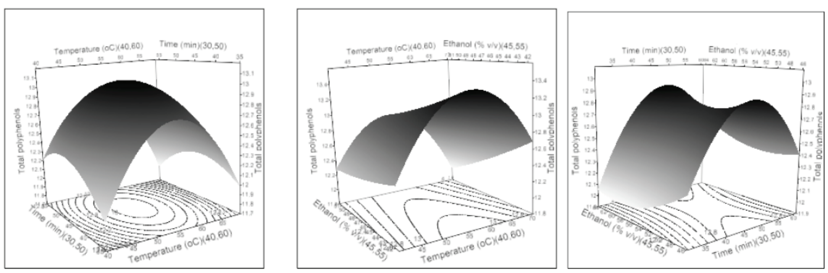
**Significant at 5% level*

Table 3. Lack of fit test for the model

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
				2.5354
Lack Of Fit	3	0.01303896	0.004346	**Prob > F**
Pure Error	32	0.05485696	0.001714	0.0743
Total Error	35	0.06789592		**Max RSq**
				0.9926

The effect of the variables and their interaction on the responses can be seen Figure 1. Figue 1a show the effect of the interaction of extraction time and extraction temperature on the total polyphenols contents at a fixed ethanol concentration of 0 level. Minimum polyphenols value was obtained at the lowest extraction time and reached the maximum value at 45 min of extraction time in the fixed extraction temperature of 55^0C. Figure 2b shows the effect of the interaction of ethanol concentration and extraction temperature on the polyphenols contents at a fixed extraction time of 0 level. Polyphenols value was increased slightly by decreasing ethanol concentration from 55% to 45% and reached the maximum value at the lowest ethanol concentration in the fixed extraction time of 55oC. As shown in Figure 1c, when extraction temperature was fixed at 0 level, minimum polyphenols value was obtained at the lowest extraction time and reached the maximum value at 45% of ethanol concentration in the fixed extraction time of 45 min. Moreover, we have found that extraction temperature (X_1) was the most significant factor affecting the responses at the level of $p < 0.01$.



a) b) c)

Figure 1. Response surface plots for the effects of extraction temperature, time and ethanol concentration on total polyphenols contents of byproducts of oolong tea extracts. **a)** extraction time and temperature;**b)** ethanol concentration and extraction temperature;**c)** ethanol concentration and extraction time.

Optimum process parameters achieved by maximizing total polyphenols content. As shown in Table 4, the predicted optimal conditions for ultrasonic extraction were found at 45% ethanol, 45.38 min extraction time and 55.04C extraction temperature. In the predicted optimal conditions, the experimental yield of polyphenols was 13.25%.

In order to verify the accuracy of the model for predicting maximal yield, we performed actual experiments using the optimized extraction conditions. The experimental values were very close to the predicted ones. This indicated that the optimization achieved in the present study was reliable. In general, effective separation of antioxidants from a complex plant matrix is a difficult procedure due to degradation of antioxidants and co-extraction of other various compounds, which are undesirable in the antioxidant extract. High extraction temperatures can increase the yield of tea polyphenols because the cell walls of the green tea leaves become more permeable to the solvent and to the constituents (Thakore, 1990; Zhao et al., 2007). However, the polyphenols can

Table 4. Optimum conditions and the predicted and experimental value of responses at the optimum conditions.

Variables	Optimum conditions (predicted)	Modified conditions (actual)
Temperature (°C)	55.04	55
Extraction time (min)	45.38	45
Ethanol (%)	45	45
Total polyphenols (%)	13.25 ± 0.04	13.27 ± 0.15

also be subject to degradation and epimerization when the extraction is conducted at too high temperatures (Qin et al., 2002). This epimerization is undesirable because a large amount of the most important catechin, EGCG, is transformed into GCG (Harbowy and Balentine, 1997). Conversely, extraction at low temperatures is desirable to avoid these changes, while the efficiency of the extraction is low. Thus, it was necessary to add some organic solvent to the water to improve the efficiency of extraction of polyphenols from tea. The most widely used solvents for extracting phenolic compounds are methanol, ethanol, acetone, and their water mixtures. Especially, ethanol and water mixtures are commonly used for the extraction of phenols from plant materials. This is due to the wide range of phenols that the aqueous ethanol mixtures can In order to verify the accuracy of the model for predicting maximal yield, we performed actual experiments using the optimized extraction conditions. The experimental values were very close to the predicted ones. This indicated that the optimization achieved in the present study was reliable. In general, effective separation of antioxidants from a complex plant matrix is a difficult procedure due to degradation of antioxidants and co-extraction of other various compounds, which are undesirable in the antioxidant extract. High extraction temperatures can increase the yield of tea polyphenols because the cell walls of the green tea leaves become more permeable to the solvent and to the constituents (Thakore, 1990; Zhao et al., 2007). However, the polyphenols can also be subject to degradation and epimerization when the extraction is conducted at too high temperatures (Qin et al., 2002). This epimerization is undesirable because a large amount of the most important catechin, EGCG, is transformed into GCG (Harbowy and Balentine, 1997). Conversely, extraction at low temperatures is desirable to avoid these changes, while the efficiency of the extraction is low. Thus, it was necessary to add some organic solvent to the water to improve the efficiency of extraction of polyphenols from tea. The most widely used solvents for extracting phenolic compounds are methanol, ethanol, acetone, and their water mixtures. Especially, ethanol and water mixtures are commonly used for the extraction of phenols from plant materials. This is due to the wide range of phenols that the aqueous ethanol mixtures can dissolve and ethanol mixtures are acceptable for human consumption models (Turkmen et al., 2006).

3.2 Effect of particle size on effective ultrasound

Figure 2 shows the increase in the amount of polyphenols extracted using ultrasound expressed as the ratio R calculated from equation (1) at different particle size of byproducts oolong tea. The influence of particle size on the effective ultrasound was studied with four sizes of byproducts oolong tea, at 55^0C, 45 min, solid-solvent ratio 1:25, and ethanol 45% as solvent (ultrasonic input power 250 W, 40 kHz). A increase of effective ultrasound rate withthe increase of particle size was observed. Such influence is logical since the contact surface decreases and the pore diffusion path increases with particle size increasing. From the extraction curves one can conclude that the concentration of polyphenols was not exactly the same in the fractions of different particle size.

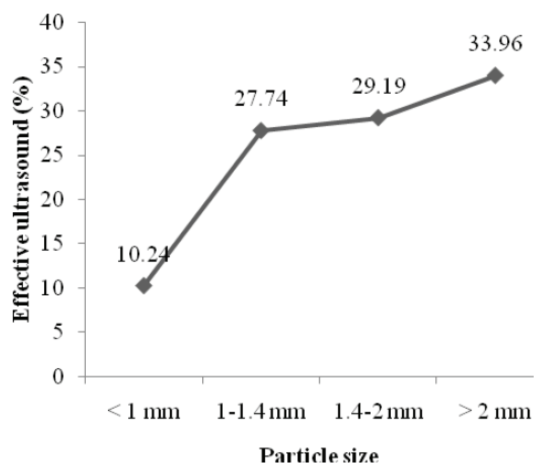


Figure 2. Effect of particle size on effective ultrasound of extraction of polyphenols from byproducts oolong tea.

In fact, ultrasound incites a formation of tiny bubbles subjected to fast adiabatic compressions and expansions, which provoke local rise of temperatures and pressures within them. Sonicated tissues absorb an extra volume of solvent. During sonication, the cavitation process provokes a swelling of the cells, solvent uptake, and an enlargement of the pores of the cell walls, which allow higher diffusivity across the cell walls. Enhanced extraction yields obtained at ultrasound assistance could also be attributed to the fact that the sonication could incite a breakdown of cell walls and facilitate the washing out of the cell content. Finally, the ultrasound assistance allowed minimizing of the extraction time. For all studies of water extraction, 15 min of ultrasound assistance gave better extraction yields than 60 min of extraction without ultrasound (d'Alessandro et al., 2012). 3.3. Comparison with diffrent extraction methods Total polyphenols extracted from byproducts of oolong tea using UAE (40 kHz, power 250W) under the optimal conditions was used to compare with the one extracted by an organic solvent, microwase assisted extraction method (MAE) (Pham et al., 2005; Pan et al., 2006) and 24 hours douse extraction in Table 5. Extraction yield when using MAE was 14.69%, higher than using UAE (13.19%). However, MAE method was limited because of problems extraction equipment and uncontrolled temperature. Consequently, UAE is suitable method to extract polyphenols from tea for further investigation.

Table 5. Results of comparing the effects of extraction methods to total polyphenols content (EtOH 45%, solid–solvent ratio 1:25)

Extraction Methods	Total polyphenols (%)	± SD
UAE	13.19^{b}	0.15
Conventional Extraction, 55°C, 45 min	11.37^{d}	0.19
Conventional Extraction, 70°C, 60 min	12.54^{c}	0.12
MAE	14.69^{a}	0.02
24 hours douse extraction	10.25^{e}	0.17

Antioxidant activity of polyphenols from byproducts oolong tea

Three different materials of oolong tea were determined of antioxidant activity by the DPPH radical scavenging method with IC50 value (Inhibitory Concentration of 50%), vitamin C was used as a control (Table 6). IC50 is defined as the concentration of substrate that causes 50% loss of the DPPH activity. It means the higher IC50 value, the lower antioxidant activity. Reducing free radical scavenging of tea powder because of the influence of temperature during spray drying to create respective powder products from the extracts. Generally, the antioxidant capacity has often been correlated with the phenolic content.

4. Conclusion

Results showed that total polyphenols in the oolong tea extracted by ultrasound resulted the highest (13.27%) when the optimal extraction conditions were 45% v/v ethanol at 55oC for 45 minutes, solid/liquid ratio of 1:25, tea particle size ¡ 1 mm by UAE method (40 kHz, 250W). Under optimized conditions the experimental values were very close to the predicted values. Compared to the conventional shaking extraction methods, ultrasonic extraction requires

Table 6. IC50 value of the materials

Materials	IC50 (µg/ml) tea powder	IC50 (µg/ml) the extracts
Vitamin C	-	16.47
M1	25.65	15.69
M2	33.84	19.07
M3	23.69	12.02

(M1: Byproducts of oolong tea; M2: tea right after enzyme inactivation; M3: oolong tea.)

less extraction time, lower temperature and provides higher polyphenol content without a decrease of antioxidant activity. As such, it may be said that ultrasonic extraction is an effective and practical method for obtaining phenolic content from oolong. And byproducts of oolong tea were potential resources used to extract them.

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