Korean J. Chem. Eng., 35(1), 187-194 (2018)
DOI: 10.1007/s11814-017-0251-6

pISSN: 0256-1115
eISSN: 1975-7220

Optimization of extraction process for bioactive compounds from *Litsea cubeba* fruits

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(*Received 19 April 2017 • accepted 18 September 2017*)

Abstract−Response surface methodology (RSM) was applied to evaluate the extraction of active compounds from *Litsea cubeba* fruits. A central composite design (CCD) with five levels and three process parameters (extraction temperature, extraction time and ethanol concentration) was used to investigate the influence of the extraction temperature, extraction time and ethanol concentration on the multiple response variables (TPC, DPPH and ABTS assays). The results of the ANOVA analysis show that the quadratic term of the ethanol concentration was significant for all the response variables. The quadratic model was highly significant ($P<0.05$) for all the response variables. After optimizing for multi-response, the optimal conditions were determined as an extraction temperature of 42.3 °C, extraction time of 126.4 min and ethanol concentration of 51%. Moreover, the extract of *Litsea cubeba* fruit attested possessing tyrosinase inhibitory activity, with an IC_{50} value of 5,720 μg mL^{-1}. The second-order kinetic model represented the kinetic data very well. In addition, the obtained values of the effective diffusivities were in the range of $2.28\text{-}5.83\times10^{-11}$ m^2s^{-1}.

Keywords: Antioxidant, Extraction, Kinetic, *Litsea cubeba*, Response Surface Methodology

INTRODUCTION

Litsea cubeba (Lour.) Persoon is an evergreen tree in the Lauraceae family that is native to China, Indonesia, Taiwan, and other areas of Southeast Asia. The local name for *Litsea cubeba* (Lour.) Persoon is "mountain pepper" in Mandarin and "maqaw" by the Atayal aborigines in Taiwan. The extracts from the bark, leaf, root and fruit have been used in traditional Chinese medicine for the treatment of inflammation, headache, and intoxication [1,2]. Owing to its unique flavor, which resembles that of a mixture of pepper, ginger, and citrus, *Litsea cubeba* (Lour.), Persoon is used as a flavor enhancer in foods, cosmetics and cigarettes [3,4]. Moreover, *Litsea cubeba* (Lour.) Persoon has attracted much attention, and is widely used in chemical and medicinal industries because of its multiple functions. Many studies have indicated that the essential oil of *Litsea cubeba* (Lour.) Persoon possesses antimicrobial [5,6], antibacterial [7], antitermite [8], larvicidal [8], acute and genetic toxicity [9], cytotoxicity [10], antioxidant and skin-whitening activities [11-13]. Among various extraction methods, the solvent extraction process is the most widely used for the extraction of bioactive compounds from plants due to its lipid-soluble (hydrophobic) nature and mature technology [14]. According to previous research, pretreatment methods, solvent properties, solid-liquid ratio, extraction time and extraction temperature influence the extraction performance [15-18]. To study the effects of those process parameters on the extraction of bioactive contents, as well as the antioxidant capacity, preliminary tests were carried out and response surface methodology (RSM) was applied. RSM is a powerful tool. The most popular design of the RSM is the central composite design (CCD) due to its simple structure and efficiency in evaluating various process variables [19]. However, there is little information on the optimization of solvent extraction involving the fruits of *Litsea cubeba* (Lour.) Persoon. Therefore, our objective was to study the antioxidant ability optimization of the *Litsea cubeba* fruit extraction process. In addition, the kinetic assay of *Litsea cubeba* fruits extraction was also analyzed to determine the efficacy of the solid-liquid extraction process. These fundamental results will provide useful information for the extraction process design.

MATERIALS AND METHODS

1. Materials

Litsea cubeba fruit was purchased from a local supermarket in Hualien County, Taiwan. The sample was ground and sieved to a fine powder (average particle diameter 0.5 mm) and stored at 4 °C for further analysis. All extraction solvents were of HPLC grade and used without further purification. Acetone (99.98%), methanol (>99%), ethanol (99.8%), sodium carbonate (Na_2CO_3) (99.5%), dimethyl sulfoxide (DMSO, 99.98%), L-Tyrosine, phosphate buffered saline (pH 6.8) and Folin-Ciocalteu's reagent (2.0 N) were purchased from Fisher Chemical, USA. Gallic acid (98%), n-hexane (>99%) and potassium persulfate (99%) were supplied by Acros, USA. DPPH (2,2-diphenyl-1-picrylhydrazyl) (95%) and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) (≥98%) were purchased from Alfa Aesar, USA.

2. Extraction of *Litsea cubeba* Fruit

In this study, 5 g of powder were mixed with the desired solid-liquid (SL) ratio of particular solvent concentration (Table 1), and then incubated in the shaker at the desired temperature at a constant rotation speed (100 rpm). After an appropriate amount of time, the extracts were removed from the shaker, and the extracts were filtered through Whatman No. 1 using a vacuum pump (KNF

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Table 1. Original and coded values of the process parameters and CCD design matrix

Independent variables	Coded	Factor levels				
		−1.68	−1	0	1	1.68
Extraction temperature (°C)	X_1	30	34	40	46	50
Extraction time (min)	X_2	30	60.4	105	149.6	180
Ethanol concentration (%)	X_3	0	20.3	50	79.7	100

Run	Independent variables			Experimental values		
	X_1	X_2	X_3	TPC (mg GAE g^{-1})	DPPH (%)	ABTS (%)
1	45.95	60.40	79.73	20.0588	17.3913	10.3597
2	45.95	149.60	20.27	21.8235	20.2899	11.3669
3	30.00	105.00	50.00	27.1176	4.71014	10.9353
4	34.05	149.60	20.27	28.8824	8.69565	8.48921
5	40.00	105.00	50.00	32.4118	25.7246	14.1007
6	40.00	105.00	50.00	29.4706	40.5797	15.8273
7	45.95	149.60	79.73	21.2353	23.1884	11.7986
8	50.00	105.00	50.00	24.7647	27.5362	13.9568
9	34.05	60.40	79.73	19.4706	12.6812	10.0719
10	34.05	60.40	20.27	28.2941	11.9565	11.0791
11	40.00	105.00	50.00	28.8824	20.6522	15.1079
12	45.95	60.40	20.27	6.52941	6.15942	9.78417
13	40.00	30.00	50.00	28.2941	23.5507	10.7914
14	40.00	105.00	50.00	37.7059	26.4493	14.6763
15	40.00	105.00	50.00	24.7647	23.913	15.6835
16	40.00	105.00	50.00	31.8235	25.3623	16.4029
17	40.00	180.00	50.00	47.7059	30.4348	14.6763
18	40.00	105.00	100.00	16.5294	11.2319	10.3597
19	40.00	105.00	0.00	20.0588	6.52174	12.8058
20	34.05	149.60	79.73	14.7647	16.3043	8.48921

LABOPORT®, Germany) for 2-3 min. The clear extract was concentrated with a rotary evaporator (Rotavapor R-3, Buchi, Switzerland) at 40 °C to a constant mass for further analysis.

3. Total Phenolic Content (TPC) Analysis

A previously proposed method [20] was used for TPC determination of the extract. The extract (1 mg mL^{-1}) was diluted and a 50 µl sample was mixed with 200 µl Folin-Ciocalteu's reagent and 2 ml of distilled water. After being well agitated for 5 min, 1 ml of aqueous sodium carbonate 15% was added and vortexed at room temperature (rt) for 2 h in the dark. A wavelength of 760 nm was recorded on a spectrophotometer (Genesys™ 10S UV-VIS, Germany), and the TPC was quantified with a calibration equation using gallic acid as a standard. TPC was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE g^{-1}).

4. Antioxidant Capacity Analysis

The method described by Shimada et al. [21] was employed with some modifications. In brief, the diluted extract 100 µl was mixed well with 1.9 ml of 99% methanol and 1 ml of 0.1 mM DPPH solution. An absorbance of 517 nm was measured after the mixture settled at rt in the dark for 30 min. Radical scavenging activity was measured using the absorbance of the control (A_c) as the DPPH solution without the extract, against the absorbance of the sample with the extract (A_s), with the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{A_c - A_s}{A_c}\right) \times 100\% \quad (1)$$

A solution of ABTS radical cations ($ABTS^+$) was prepared according to the method of Re et al. [22] and was diluted with 99.5% ethanol to an absorbance of 0.7±0.05 at 734 nm for the absorbance of the control (A_c) to determine the ABTS radical scavenging capacity of the extract. Then, 1ml of diluted $ABTS^+$ solution was mixed with 20 µl of diluted extract in the dark at rt for 6 min; the absorbance (A_s) was recorded at 734 nm. The ABTS radical scavenging capacity of the extract was determined by using the following equation:

$$\text{ABTS radical scavenging capacity (\%)} = \left(\frac{A_c - A_s}{A_c}\right) \times 100\% \quad (2)$$

5. Tyrosinase Inhibitory Assay

The tyrosinase inhibitory assay described by Kubo and Kinst-Hori [23] was performed with slight modifications. The extract was dissolved in 5 ml of DMSO and diluted with phosphate buffered saline (pH 6.8); 1.8 ml of the sample was mixed well with 2 ml of phosphate buffered saline solution and 2 ml of L-Tyrosine. After the mixture was incubated at 27 °C for 30 min, 200 µL of mushroom tyrosinase (1,000 U mL^{-1}) was added in the dark for

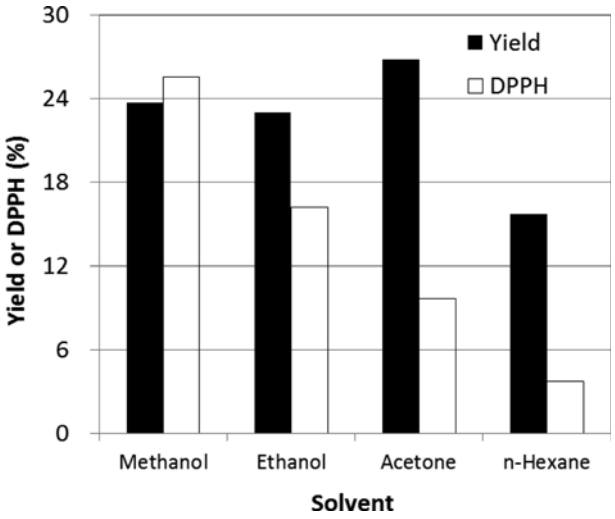

Fig. 1. Effect of various solvents on extraction yield and DPPH radical scavenging activity.

10 min. Then, an absorbance at 475 nm was recorded. Arbutin was used as a reference. The percentage of inhibition was determined by using the following formula [12]:

$$\text{Inhibition}\ (\%) = \left(\frac{A_s - A_{blank}}{A_c}\right) \times 100 \quad (3)$$

where A_s is the absorbance of the test sample with tyrosinase; A_{blank} is the absorbance of the test sample without tyrosinase; and A_c is the absorbance of tyrosinase solution without test sample.

6. Experimental Design and Statistical Analysis

6-1. Preliminary Test

Since many process parameters can influence the extraction performance, preliminary tests must be carried out to determine the reduction of the control variables. Initially, four common solvents--methanol, ethanol, acetone, and n-hexane--were used as the extraction solvents (solid-liquid ratio=1 : 30) at 50 °C for 60 min in an incubated shaker to test the extraction yield and DPPH radical scavenging activity of the *Litsea cubeba* fruits. The experimental results are in Fig. 1; it shows a higher extraction yield and DPPH radical scavenging activity of *Litsea cubeba* fruits when methanol was used as the extraction solvent. Subsequently, methanol was chosen to study the influences of the solid liquid ratio and extraction temperature in the extraction process. The experimental results are shown in Fig. 2. The extraction yield was increased with the increased solid-liquid ratio and extraction temperature. Beyond a solid-liquid ratio of 1 : 30, the changes of the extraction yield and DPPH radical scavenging activity were not significant. Conversely, the DPPH radical scavenging activity decreased with the increased solid ratio and extraction temperature. When the extraction temperature was over 50 °C, the DPPH radical scavenging activity approached greater stability. According to Fig. 1, the extraction performance of ethanol was less efficient, but still comparable to that of methanol. In addition, ethanol is preferred over methanol as an extraction solvent because of its environmentally-friendly nature [24]. Therefore, the extraction solvent (ethanol) and a solid liquid ratio of (1 : 30) were selected, and the extraction temperature range of 30-50 °C was determined for the optimization analysis of the antioxidant extraction from *Litsea cubeba* fruits.

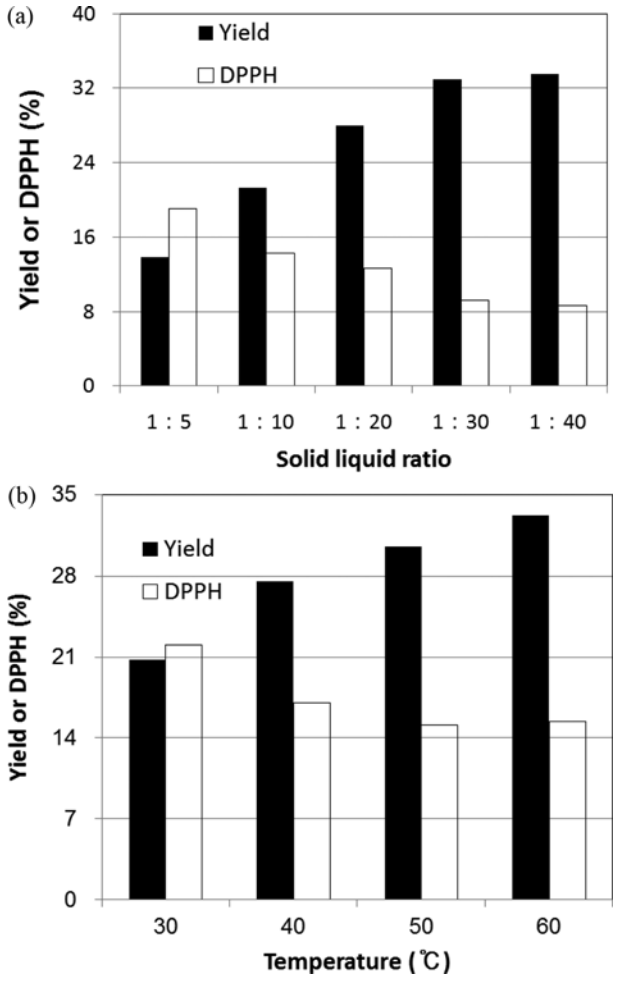

Fig. 2. Effect of (a) solid liquid ratio, (b) extraction temperature on extraction yield and DPPH radical scavenging activity.

6-2. Central Composite Design and Statistical Analysis

A central composite design (CCD) with five levels ($-\alpha$, -1, 0, 1, $+\alpha$) and three process parameters (extraction temperature, extraction time, and ethanol concentration) was used to investigate the influence of extraction temperature, extraction time, and solvent concentration on the multiple response variables (TPC, DPPH and ABTS assays). Furthermore, the highest extraction yields of TPC and antioxidant capacity (DPPH and ABTS assays) were studied, and the optimal extraction conditions were determined. The original coded values of the process factors and CCD design matrix are tabulated in Table 1. After 20 experimental runs using the CCD design matrix, the corresponding experimental data were regressed using Design Expert software (version 10.0). The effects of each process parameter and interaction were studied via analysis of variance (ANOVA) for each response variable. The regression results of the CCD experimental design matrix, response surface statistics and plots were performed using the Design Expert program. All of the experiments were carried out in triplicate, and the response variables obtained were averaged.

7. Extraction Kinetics Theory

The extraction kinetics models were used to quantify the efficacy of the solid-liquid extraction process. The general second-order kinetic model can be written as [25]:

$$\frac{dC_t}{dt} = k(C_e - C_t)^2 \quad (4)$$

where k is the second-order rate constant; C_e is the equilibrium extraction yield; and C_t is the extraction yield at any time t. The integrated rate law for a second-order extraction under the conditions C_t=0 at t=0 and C_t=C_t at t=t can be obtained via Eq. (5):

$$C_t = \frac{C_e^2 kt}{1 + C_e kt} \text{ or } \frac{t}{C_t} = \frac{1}{kC_e^2} + \frac{t}{C_e} \quad (5)$$

The equilibrium extraction yield (C_e) and the second-order rate constant (k) can be calculated by plotting the (t/C_t) versus (t) and then determining the slope ($1/C_e$) and the intercept ($1/kC_e^2$) [26].

Assuming no change in the effective diffusivity with solute concentration, and considering that particles have a spherical shape and concentration differences only relevant in a radial direction, Fick's second-law can be used to describe the non-steady state diffusion of an extraction process [27]. The analytical solution can be expressed as the following equation:

$$\frac{C_e - C_t}{C_e} = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-n^2\frac{\pi^2 D_{eff} t}{r^2}\right) \quad (6)$$

where D_{eff} is the effective diffusivity (m^2s^{-1}), and r is the ratio of diffusion (m). According to Schwartzberg [28], only the first term of the series solution can be used with a little error where the external resistance is negligible. Therefore, Eq. (6) can be simplified and the effective diffusivity (D_{eff}) can be calculated graphically:

$$\frac{C_e - C_t}{C_e} = \frac{6}{\pi^2}\exp\left(-\frac{\pi^2 D_{eff} t}{r^2}\right) \quad (7)$$

RESULTS AND DISCUSSION

1. Model Fitting

RSM is an empirical modeling technique used to investigate the relationship between the process variables and the response variables [29,30]. The CCD design of RSM was used to study the effects of extraction temperature (30-50 °C), extraction time (30-180 min) and ethanol concentration (0-100%) on the TPC and antioxidant capacity of *Litsea cubeba* fruits. The experimental design and results are presented in Table 1. Various models, such as the linear, quadratic and cubic models, were fitted to the experimental data to obtain the regression coefficients by using the least square method. The quadratic model was highly significant (P<0.05) for these three response variables, as shown in Table 2. Therefore, the quadratic models in coded factors for TPC, DPPH, and ABTS were constructed:

$$TPC = 31.07 - 1.88X_1 + 3.3X_2 - 1.17X_3 + 2.57X_1X_2 + 4.49X_1X_3 - 2.43X_2X_3 - 3.23X_1^2 + 1.04X_2^2 - 5.93X_3^2 \quad (8)$$

$$DPPH = 27.18 + 4.08X_1 + 2.33X_2 + 2.22X_3 + 2.45X_1X_2 + 0.72X_1X_3 - 0.18X_2X_3 - 4.34X_1^2 - 0.5X_2^2 - 6.9X_3^2 \quad (9)$$

Table 2. ANOVA analysis results of the quadratic model

Source	DF (Degree of freedom)	SS (Sum of squares)	*F* value	*P* value
TPC				
Model	9	1135.16	3.76	0.0254
X_1	1	48.45	1.44	0.2572
X_2	1	148.27	4.42	0.0618
X_3	1	18.59	0.55	0.4737
X_1X_2	1	52.98	1.58	0.2374
X_1X_3	1	160.94	4.80	0.0533
X_2X_3	1	47.10	1.40	0.2635
X_1^2	1	149.92	4.47	0.0606
X_2^2	1	15.53	0.46	0.5117
X_3^2	1	506.59	15.1	0.0030
Lack of fit	5	242.30	2.60	0.1589
R^2=0.7719				
Adj R^2=0.5666				
DPPH				
Model	9	1307.04	4.03	0.0202
X_1	1	227.83	6.32	0.0307
X_2	1	74.36	2.06	0.1814
X_3	1	67.60	1.88	0.2008
X_1X_2	1	47.85	1.33	0.2760
X_1X_3	1	4.20	0.12	0.7399
X_2X_3	1	0.26	$7.285*10^{-3}$	0.9737
X_1^2	1	271.48	7.53	0.0207
X_2^2	1	3.56	0.099	0.7596
X_3^2	1	686.57	19.05	0.0014
Lack of fit	5	121.62	0.51	0.7616
R^2=0.7839				
Adj R^2=0.5893				
ABTS				
Model	9	106.37	12.36	0.0005
X_1	1	7.71	8.06	0.0194
X_2	1	1.04	1.09	0.3233
X_3	1	1.24	1.3	0.2844
X_1X_2	1	6.47	6.77	0.0287
X_1X_3	1	0.51	0.53	0.4849
X_2X_3	1	0.093	0.097	0.7620
X_1^2	1	19.25	20.13	0.0015
X_2^2	1	38.48	40.25	0.0001
X_3^2	1	30.44	31.84	0.0003
Lack of fit	4	5.1	1.82	0.2628
R^2=0.9252				
Adj R^2=0.8503				

$$ABTS = 15.33 + 0.75X_1 - 0.34X_2 - 0.3X_3 + 0.9X_1X_2 + 0.25X_1X_3 + 0.11X_2X_3 - 1.18X_1^2 - 2.14X_1^2 - 1.49X_3^2 \quad (10)$$

ANOVA is a statistical technique for analyzing the significance of each coefficient of the term in the quadratic models [31]. The ANOVA results show that the quadratic term of ethanol concentration was significant for all the response variables. The linear and

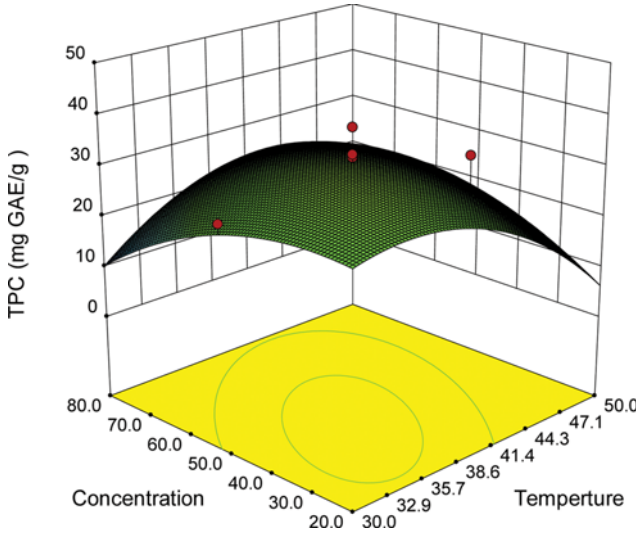

Fig. 3. Response surface plot showing the effects of temperature and ethanol concentration on TPC.

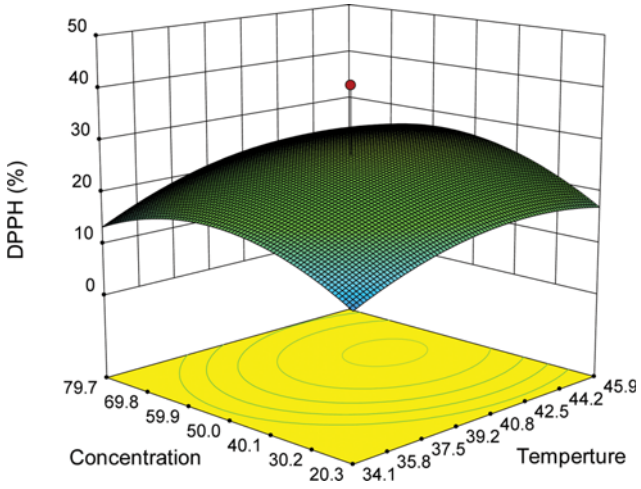

Fig. 4. Response surface plot showing the effects of temperature and ethanol concentration on the DPPH radical scavenging activity.

quadratic terms of temperature were also significant for DPPH and ABTS. In addition, the quadratic terms of extraction time and interaction between extraction temperature and time for ABTS were also significant. These statistical analyses also revealed that the most important control variable for the bioactive compounds extraction from *Litsea cubeba* fruits was ethanol concentration. The ANOVA analysis results of the quadratic models displayed a small *P* value, high *F* value, R-squared (R^2), adjusted R-squared (Adj. R^2) and an insignificant lack of fit (P>0.05). These results show that the quadratic model was significant, and can be applied to represent the extraction process between response variables and control factors [31,32].

2. Effects of Process Parameters on Response Variables

To display the influence of two process parameters on the extraction process, we used response surface plots (Figs. 3-5). The polarity of the solvent is an important factor in the extraction of active compounds by solubilization. Since polyphenols have a wide range of solubility, a mixed solvent may be more effective than any single solvent in extracting bioactive components from a solid matrix [33]. The presence of water will lead to the increased polarity of the ethanol solution, and also increase the contact surface area between the plant matrix and the solvent by the plant material swelling [34]. As indicated in Eq. (8), the extraction time in linear (X_2) and quadratic (X_2^2) terms had positive effects on TPC extraction. The interactive effects of extraction temperature and time (X_1X_2) and extraction temperature and ethanol concentration (X_1X_3) also showed positive effects. Fig. 3 depicts the effects of extraction temperature and ethanol concentration on TPC from *Litsea cubeba* fruits with a maximum pattern at a fixed time of 105 min. Furthermore, temperature control is another pivotal factor in the extraction process. The temperature exhibited a similar effect to that of ethanol concentration. When the temperature increased, the diffusion rate of the liquid solvent into the solid matrix was accelerated, and the kinetics of desorption of the active compounds from the matrix was increased [35]. However, a higher extraction temperature will increase the possibility of thermal degradation.

The effects of ethanol concentration and extraction temperature on the DPPH radical scavenging activity of *Litsea cubeba* fruits is shown in Fig. 4. Eq. (9) indicates that the linear effects of ex- traction temperature (X_1) and ethanol concentration (X_3) were positive, whereas their quadratic terms were negative, which contributed to a curved rise in DPPH radical scavenging activity. Based on the response surface plot shown in Fig. 4, the DPPH radical scavenging activity increased significantly with the increased extraction temperature. Accordingly, the DPPH radical scavenging activity was enhanced by increasing the extraction temperature from 20 °C to 45 °C with an increase of ethanol concentration, because more antioxidant compounds diffused into the solvent at higher temperatures [36]. However, a slight decrease of the DPPH radical scavenging activity at higher temperature was observed with the increase of ethanol concentration from 50% to 80%.

The entire interaction (X_1X_2, X_1X_3, and X_2X_3) between the pro-

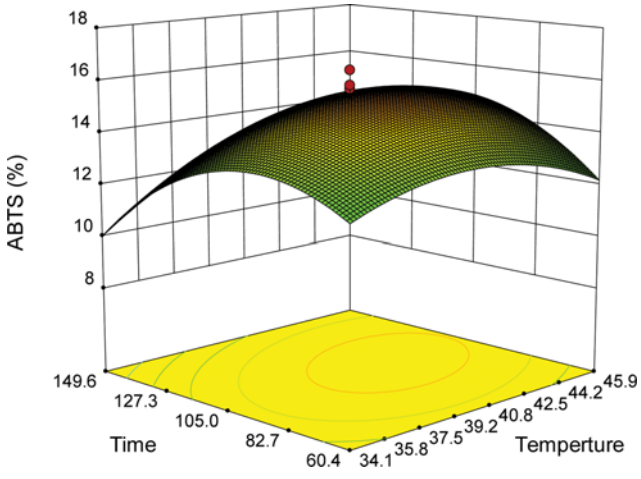

Fig. 5. Response surface plot showing the effects of temperature and time on ABTS radical scavenging capacity.

cess parameters and extraction temperature in linear terms (X_1) had a positive effect on the ABTS radical scavenging capacity, as indicated in Eq. (10). Fig. 5 displays the effects of extraction temperature and time on ABTS radical scavenging capacity at a fixed ethanol concentration of 50%. The interaction (X_1X_2) between extraction temperature and time for ABTS radical scavenging capacity was also significant (P<0.05 from Table 2), as illustrated by the response surface plot. Both control variables represented the strong effect on the ABTS radical scavenging capacity of *Litsea cubeba* fruits. The ABTS radical scavenging capacity was increased by increasing the extraction temperature as well as the time, as per specified conditions. At higher extraction temperatures, the ABTS radical scavenging capacity increases with the increased extraction time, but further increase in extraction time leads to a decrease in ABTS radical scavenging capacity. Therefore, extraction temperature is not an independent process parameter, and the interaction effect of extraction temperature and time should be considered.

3. Determination of Optimal Conditions and Model Verification

To obtain the maximum responses, a numerical optimization technique was applied by using Design Expert software; the extraction under optimal conditions was conducted three times for each response assessment. At the optimal conditions (extraction temperature 42.3 °C, time 126.4 min and ethanol concentration 51%), the predicted TPC, DPPH and ABTS values under the optimal conditions were 32.119 mg GAE g^{-1}, 29.655% and 14.945%, respectively, which were close to the experimental values: 36.53± 0.228 mg GAE g^{-1}, 28.76±0.560% and 13.7±0.741%, respectively. Therefore, the fitted quadratic models in this study can be regarded as suitable to represent the extraction of *Litsea cubeba* fruits.

4. GC-MS Analysis and Tyrosinase Inhibitory Effect of the Extract

The extraction under optimal conditions was conducted and the compounds present were identified by GC-MS. The major compounds with their retention time (RT) and compositions are presented in Table 3. The main components in the extract are aldehydes (35.70%), alkenes (20.57%), ketones (12.71%), esters (11.43%) and alcohols (5.19%). Some studies have proven that aldehydes (citral and isogeranial) and alcohols (γ-terpineol) have antioxidant ability [12,37-39]. Moreover, the tyrosinase inhibitory

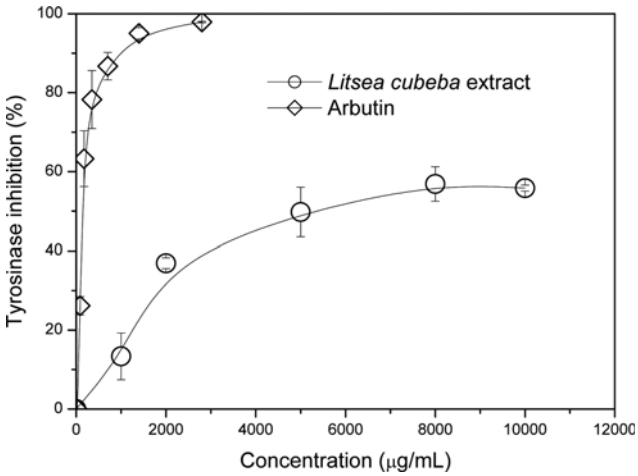

Fig. 6. The tyrosinase inhibitory activity of different concentrations of the extract and arbutin.

effect of the extract was determined. The key enzyme to catalyze the tyrosine to melanin is tyrosinase, which is widely distributed in the human body. Therefore, tyrosinase inhibitory activity, which is important for cosmetic products, was examined for the extract under optimal conditions, as shown in Fig. 6. The extract of *Litsea cubeba* fruit attested as having tyrosinase inhibitory activity, with an IC_{50} value of 5,720 μg mL^{-1} contributed by the citral component [12]. However, the tyrosinase inhibitory activity of *Litsea cubeba* fruit is lower than the reference sample, Arbutin (IC_{50}= 166 μg mL^{-1}).

5. Extraction Kinetic Analysis

All the extractions of *Litsea cubeba* fruits for kinetic assays involved using the optimal conditions mentioned in Section 3.3, with changes to the extraction temperatures (30, 45 and 60 °C) and ethanol concentrations (30%, 60%, and 90%), respectively. Samples were taken and analyzed (the extraction yield and TPC) at predetermined intervals. To more specifically express the TPC variations with extraction times, the TPC was expressed as milligrams of gallic acid equivalent per gram of dry fruit (mg GAE g^{-1}DF). The second-order law was applied to the extraction process and

Table 3. The major compounds of the extract of *Litsea cubeba* fruits

No.	RT	Compounds	Composition (%)
1	14.45	Citral	32.14
2	38.33	Cryptomerione	12.71
3	26.23	6,7-Dimethyl-1,2,3,5,8,8a-hexahydronaphthalene	8.18
4	23.00	Bicyclogermacrene	7.66
5	40.40	Ethyl hexadecanoate	7.23
6	39.04	4,8,12-Trimethyltridec-3-enoic acid, methyl ester	4.81
7	20.00	Caryophyllene	4.73
8	34.37	Ethyl linoleate	4.20
9	11.12	Isogeranial	3.56
10	17.40	γ-Terpineol	3.01
11	18.08	Verbenol	2.18
12	-	Others	9.56

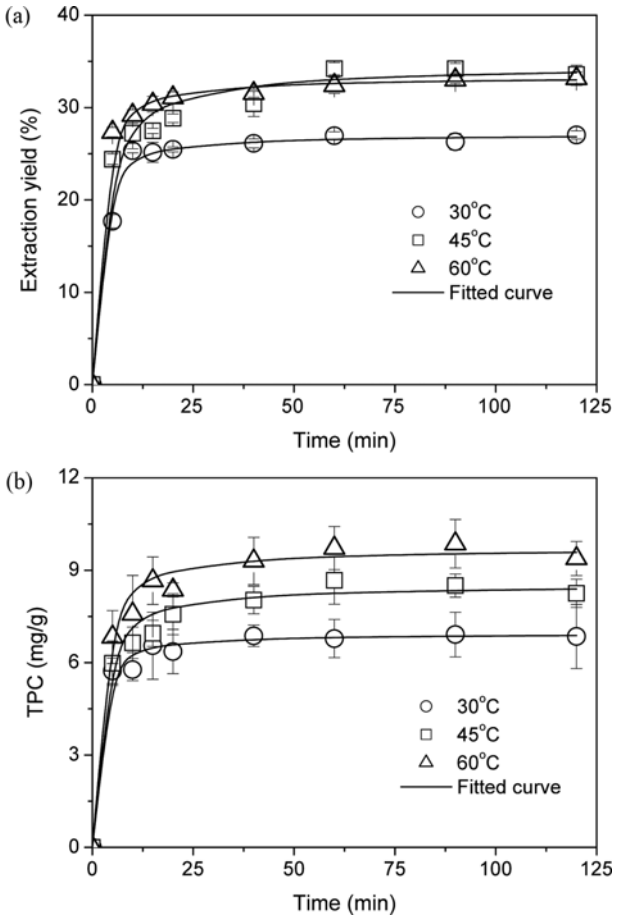

Fig. 7. Extraction of *Litsea cubeba* fruits at different time intervals and extraction temperatures: (a) Extraction yield; (b) TPC.

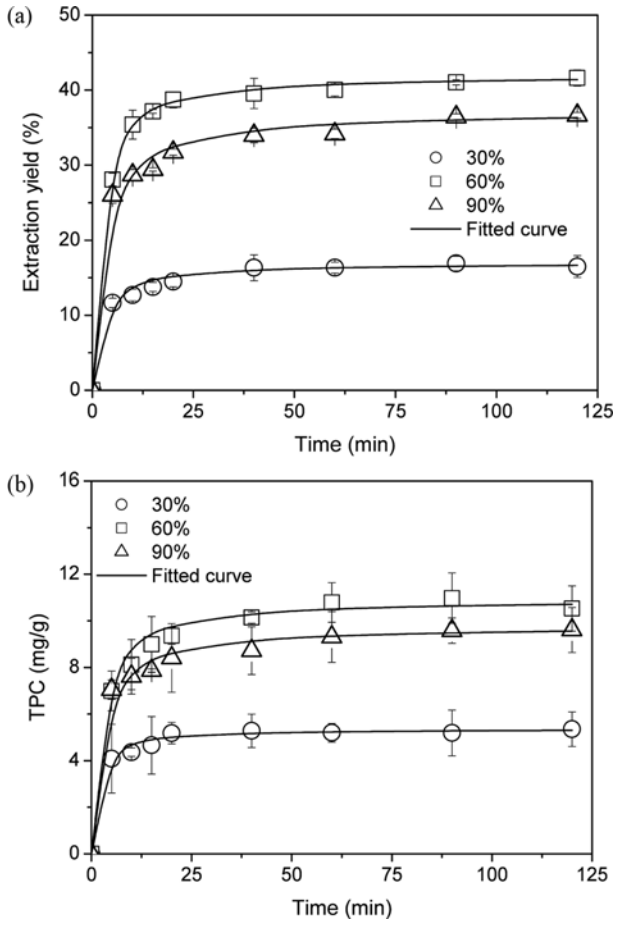

Fig. 8. Extraction of *Litsea cubeba* fruits at different time intervals and ethanol concentrations: (a) Extraction yield; (b) TPC.

was found to be the best regression model [40]. The extraction kinetics data were fitted with the second-order kinetic model (Figs. 7-8); the kinetic parameters derived from these models are listed in Table 4. The second-order kinetic model for the different solvents and temperatures was characterized by a coefficient of determination (R^2) close to unity. From Table 4, the parameter C_e increased as the temperature increased from 30 °C to 45 °C, and then decreased. Meanwhile, the parameters k and D_{eff} were just the opposite. Similarly, parameter C_e increased as the ethanol concentration increased from 30% to 60%, and then decreased. At the same time, the parameter D_{eff} of the extraction yield had the same trend, but parameters k and D_{eff} of TPC decreased with an increase in ethanol concentration. Moreover, the values of the effective diffusivities were in the range of 2.28-5.83×10^{-11} m^2s^{-1}, which is com-

Table 4. Kinetic parameters determined using the optimal conditions at different extraction temperature and ethanol concentration

Kinetic parameters	Temperature (°C)			Ethanol concentration (%)		
	30	45	60	30	60	90
Extraction yield						
k ($g \cdot g^{-1}min^{-1}$)	0.0289	0.0114	0.0238	0.0264	0.0134	0.0097
C_e ($g \cdot g^{-1}$)	27.1003	34.4828	33.3333	16.9492	42.0168	37.1747
D_{eff} ($m^2 \cdot s^{-1}$)×10^{-11}	5.83	3.30	3.30	3.30	4.56	3.30
R^2	0.9995	0.9981	0.9998	0.9990	0.9996	0.9986
TPC						
k ($g \cdot mg^{-1}min^{-1}$)	0.1413	0.0655	0.0638	0.1384	0.0400	0.0398
C_e (mg GAE·g^{-1}DF)	6.9348	8.5106	9.7087	5.3562	10.9170	9.7656
D_{eff} ($m^2 \cdot s^{-1}$)×10^{-11}	5.32	3.80	3.80	4.56	2.54	2.28
R^2	0.9997	0.9984	0.9982	0.9994	0.9983	0.9989

parable to 1.23-1.50×10^{-11} m^2s^{-1} found for the polyphenol extraction with 60% ethanol [41] and 3.49-9.28×10^{-11} m^2s^{-1} for ultrasound-assisted TPC extraction [42].

CONCLUSIONS

The CCD of response surface methodology was applied to maximize the extraction of TPC, DPPH radical scavenging activity and ABTS radical scavenging capacity of *Litsea cubeba* fruits. In general, the active compounds increased with an increase in the extraction temperature; however, higher extraction temperature and more time also increased the possibility of thermal degradation. The ANOVA analysis results showed that the quadratic term of ethanol concentration was significant for all the response variables. The response surface plots were used to evaluate the interactive effect of the process parameters on the response variables. Various models were fitted to the experimental data to construct a suitable model. The quadratic model was highly significant ($P<0.05$) for all the response variables. After optimizing for multi-response, the optimal conditions (extraction temperature 42.3 °C, time 126.4 min and ethanol concentration 51%) were determined. Under optimal conditions, the experimental values were close to the predicted values. The kinetic extraction of *Litsea cubeba* fruits at optimal conditions with changes to the extraction temperatures (30 °C, 45 °C and 60 °C) and ethanol concentration (30%, 60% and 90%) were examined. A second-order kinetic model was applied to fit the kinetic data and was represented very well. Moreover, the values of the effective diffusivities were in the range of 2.28-5.83×10^{-11} m^2s^{-1}, which is comparable to the published data in the literature.

ACKNOWLEDGEMENTS

Financial support from the Ministry of Science and Technology, ROC, through Grant MOST 104-2221-E-027-102 is gratefully acknowledged. The anonymous reviewers are also appreciated for their comments.

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