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Mechanochemical-Assisted Extraction and Antioxidant Activities of Kaempferol Glycosides from *Camellia oleifera* Abel. Meal

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ABSTRACT: A mechanochemical-assisted extraction (MCAE) method was proposed and investigated for the fast extraction of two kaempferol glycosides (kaempferol-3-*O*-[2-*O*-*β*-D-galactopyranosyl-6-*O*-α-L-rhamnopyranosyl]-*β*-D-glucopyranoside and kaempferol-3-*O*-[2-*O*-*β*-D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl]-*β*-D-glucopyranoside) from *Camellia oleifera* Abel. meal. The effects of operating parameters in terms of NaOH content, grinding time, extraction time, and ratio of solution to solid were evaluated by means of response surface methodology (RSM). Under the optimal conditions with a ratio of material to NaOH of 20:1 (g/g), a milling time of 15 min, and a ratio of solution to solid of 20:1 (mL/g) for 60 min, the maximum extraction yields of the two kaempferol glycosides reached 13.34 and 13.83%, respectively. The antioxidant activity of kaempferol glycosides extract was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and ferric thiocyanate (FTC) assay. Compared with the heat reflux extraction (HRE) method, the yield and the antioxidant activities of the extracts from MCAE with water as solvent were higher and stronger.

KEYWORDS: *Camellia oleifera* Abel. meal, mechanochemical-assisted extraction, kaempferol glycosides, antioxidant activities, response surface methodology

INTRODUCTION

Camellia oleifera Abel. (Theaceae) meal, which is the byproduct produced by pressing seeds during traditional oil processing, has not been fully used for a long time and causes much pollution to the environment but contains many active substances including saponins, flavonoids, and polysaccharides. It has been indicated that *C. oleifera* Abel. possesses many benefits to health, including antioxidation, antihepatotoxic, antibacterial,[1,2] and protective effects against many diseases such as cancer, cardiovascular disease, and inflammation.[3−5] A reasonable amount of kaempferol glycosides (KG) have been found in *C. oleifera* Abel. meal, and these were determined to be kaempferol-3-*O*-[2-*O*-*β*-D-galactopyranosyl-6-*O*-α-L-rhamnopyranosyl]-*β*-D-glucopyrnoside (KG1) and kaempferol-3-*O*-[2-*O*-*β*-D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl]-*β*-D-glucopyranoside (KG2) [6] (Figure 1). Both kaempferol glycosides showed an inhibitory effect on the arachidonate 5-lipoxygenase of RBL-1 cells.[7] It is significant to extract kaempferol glycosides from *C. oleifera* Abel. meal.

A conventional organic solvent extraction method has been used to extract two kaempferol glycosides. The yields of two kaempferol glycosides extracted from green tea seed were 0.25 and 0.31%, respectively.[7,8] Although this method can obtain mostly active composition, heating processes can result in the loss or degradation of target analytes.[9] Moreover, the low selectivity and organic solvent remnant make the latter purification difficult. Mechanochemistry is a branch of chemistry used to describe the chemical and physicochemical transformation of substances during aggregation caused by mechanical energy.[10] This general definition has been formulated by Heineke[11] and is widely adopted nowadays.[12] Mechanochemical-assisted extraction (MCAE) has been frequently used in many fields of human activity, such as extractive metallurgy, crystal engineering, materials engineering, agriculture, and pharmacy,[10] and covers a wide range of important reactions, such as faster decomposition and synthesis,[13,14] polymorphic transformation,[15] and plant materials treatment.[16] MCAE as an alternative extraction method that implements mechanochemical processing to the material with solid reagent to obtain mechanochemical composites before extraction in solvent has been used to extract triterpene acids from Siberian fir needles,[17] to extract phytoecdysteroids from *Serratula coronata* L.,[18] to isolate lappaconitine from *Aconitum septentrionale* roots,[19] chondroitin sulfate from shark cartilage,[20] isofraxidin from *Eleutherococcus senticosus*,[21] etc. However, the application of MCAE on the extraction of kaempferol glycosides from *C. oleifera* Abel. meal has never been reported.

In this paper, MCAE was developed to extract KG from *C. oleifera* Abel. meal. The effects of the main operating parameters, namely, the ratio of material to solid reagent, milling time, extraction time, and ratio of solution to solid, were optimized using central composite rotatable design combined with response surface methodology (RSM). The MCAE method was compared with heat reflux extraction (HRE), and the antioxidant activities of extracts, obtained with these two extraction methods were determined by means of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and ferric thiocyanate (FTC) assay.

MATERIALS AND METHODS

Materials and Reagents. *C. oleifera* Abel. meal was provided from Datian County (Fujian, China). The raw materials were pulverized into powder by a pulverizer (DFY-500, Ding Guang Machinery Equipment Co., Ltd., Shanghai, China), defatted with mineral ether for 3 h by HRE,

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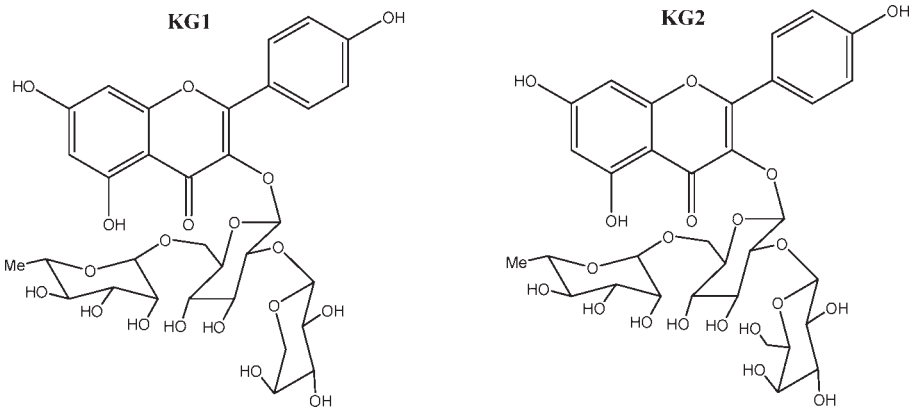

Figure 1. Molecular structures of the KG1 and KG2.

Table 1. Coded and Uncoded Levels of the Four Variables Used in MCAE of Kaempferol Glycosides

		levels[a]				
factor	symbol	−2	−1	0	1	2
ratio of material to solid reagent (g/g)	X_1	10:1	15:1	20:1	25:1	30:1
milling time (min)	X_2	5	10	15	20	25
extraction time (min)	X_3	30	45	60	75	90
ratio of solution to solid (mL/g)	X_4	15:1	20:1	25:1	30:1	35:1

[a] $x_1 = (X_1 - 20)/5$; $x_2 = (X_2 - 15)/5$; $x_3 = (X_3 - 60)/15$; $x_4 = (X_4 - 25)/5$.

and stored at 60 °C until use. The pure KG1 and KG2 of reference standards were prepared according to the method demonstrated by Li.[22] HPLC grade solvents were purchased from Tedia Co. Inc., and DPPH was supplied by Sigma-Aldrich (Steinheim, Germany). Ascorbic acid (V_C) was provided by Guangdong Guanghua Chemical Factory Co., Ltd. (Guangdong, China). Butylated hydroxytoluene (BHT) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Linoleic acid was purchased from Shanghai Runjie Chemical Reagent Co., Ltd. (Shanghai, China). All of the chemicals and reagents were of analytical grade.

Heat Reflux Extraction. According to the optimal conditions, 20.0 g of defatted sample was accurately weighed and heat-refluxed with 300 mL of 60% (v/v) aqueous ethanol for 3 h at 70 °C, and then the mixtures in tubes were centrifuged for 15 min at 4700 rpm. After the process had been repeated two times, the supernatants were combined and concentrated by rotary evaporator (EYELA N-1100, Boxun Co., Ltd., Shanghai, China) in a vacuum of 0.09 MPa at 50 °C for 30 min prior to HPLC (Agilent 1100, Agilent Technologies Co., Ltd., China) analysis.

Mechanochemical-Assisted Extraction. Twenty grams of defatted powder and different amounts of solid reagent were added into an AGO-2 high-intensity planetary activator. After several minutes of grinding, the powder was extracted with an appropriate volume of water for a short time. Then the mixture was clarified by centrifugation at 4700 rpm for 15 min. The supernatant was acidified to pH 5.0 with acetic acid and evaporated in a vacuum of 0.09 MPa at 55 °C for 40 min before HPLC analysis. The yield is expressed as the percent ratio of the mass of extracted KG1 (KG2) to the mass of *C. oleifera* Abel. meal loaded in the extraction vessel as

$$\text{yield}(\%) = m/M \times 100 \quad (1)$$

where m is the weight of KG1 (KG2) analyzed by HPLC (g) and M is the weight of *C. oleifera* Abel. meal (g).

Experimental Design. RSM is an empirical modeling technique used to estimate the relationship between a set of control experimental factors and observed results.[23,24] A central composite design (CCD) was used to determine the optimal conditions of MCAE for KG. On the basis of the single-factor experimental results, major parameters were as follows: ratio of material to solid reagent (X_1), milling time (X_2), extraction time (X_3), and ratio of solution to solid (X_4). The ranges for the variables are shown in Table 1. In this study, 30 experimental runs were employed, and experiments were performed in randomized order according to the run number as arranged by the software. The variables were coded according to the equation

$$x_i = X_i - X_0/\Delta X \quad i = 1,\ 2,\ 3,\ 4 \quad (2)$$

where x_i and X_i are the dimensionless and the actual value of the independent variable i, respectively, X_0 is the actual value of X_i at the central point, and ΔX is the step change.

Data from the CCD can be described by the second-order polynomial model[25,26]

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

where Y is the response function, β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are the coded values of the independent variables. The actual and coded levels of the independent variables used in the experimental design are shown in Table 2.

Statistical Analysis. The data from MCAE tests designed by CCD were analyzed using Design Expert 7.1.6 software (Stat-Ease, Inc.). Analyses of variance were performed by ANOVA procedure. Mean values were considered to be significantly different when $P < 0.05$.

Determination of KG1 and KG2. A Waters HPLC system was employed to determine the contents of KG1 and KG2. A SUNFIRE C_{18} column (250 mm × 4.6 mm, 5 μm) was used. The mobile phase was acetonitrile/aqueous H_3PO_4 (pH 2.8) (10:90, v/v) and was filtered through a 0.45 μm membrane filter prior to use. The injection volume was 5 μL, the flow was 1 mL/min, and the column temperature was set at 25 °C. KG could be detected by UV at 266 nm. The HPLC chromatogram of the extract from *C. oleifera* Abel. meal is shown in Figure 2. Analyses were performed at least three times, and only mean values were reported.

Scanning Electron Microscopy (SEM). The morphological alterations of dried samples with different extraction methods were observed by SEM. The samples of milled and raw *C. oleifera* Abel. meal powder were examined with a Hitachi S-4700 field scanning electron microscopy (Hitachi, San Jose, CA) under high-vacuum conditions and at an accelerating voltage of 15.0 kV.

Table 2. Coded and Real Levels of Operational Parameters and Observed Responses

	variable				factor					
run	X_1	X_2	X_3	X_4	*A* (g/g)	*B* (min)	*C* (min)	*D* (mL/g)	yield of KG1 (%)	yield of KG2 (%)
1	−1	−1	−1	−1	15:1	10	45	20:1	11.56	12.24
2	1	−1	−1	−1	25:1	10	45	20:1	12.25	12.79
3	−1	1	−1	−1	15:1	20	45	20:1	10.44	11.08
4	1	1	−1	−1	25:1	20	45	20:1	10.03	11.84
5	−1	−1	1	−1	15:1	10	75	20:1	11.58	12.31
6	1	−1	1	−1	25:1	10	75	20:1	12.31	12.81
7	−1	1	1	−1	15:1	20	75	20:1	10.05	11.11
8	1	1	1	−1	25:1	20	75	20:1	10.44	12.06
9	−1	−1	−1	1	15:1	10	45	30:1	12.01	12.65
10	1	−1	−1	1	25:1	10	45	30:1	12.45	13.02
11	−1	1	−1	1	15:1	20	45	30:1	10.13	11.05
12	1	1	−1	1	25:1	20	45	30:1	10.15	12.12
13	−1	−1	1	1	15:1	10	75	30:1	11.98	12.72
14	1	−1	1	1	25:1	10	75	30:1	12.41	13.11
15	−1	1	1	1	15:1	20	75	30:1	10.56	11.35
16	1	1	1	1	25:1	20	75	30:1	11.28	12.32
17	−2	0	0	0	10:1	15	60	25:1	10.88	12.86
18	2	0	0	0	30:1	15	60	25:1	11.66	12.98
19	0	−2	0	0	20:1	5	60	25:1	11.04	11.84
20	0	2	0	0	20:1	25	60	25:1	8.02	9.57
21	0	0	−2	0	20:1	15	30	25:1	11.93	12.67
22	0	0	2	0	20:1	15	90	25:1	10.93	12.55
23	0	0	0	−2	20:1	15	60	15:1	12.07	12.55
24	0	0	0	2	20:1	15	60	35:1	13.17	13.82
25	0	0	0	0	20:1	15	60	25:1	13.12	13.61
26	0	0	0	0	20:1	15	60	25:1	13.18	13.81
27	0	0	0	0	20:1	15	60	25:1	13.01	13.33
28	0	0	0	0	20:1	15	60	25:1	13.35	13.54
29	0	0	0	0	20:1	15	60	25:1	13.28	13.75
30	0	0	0	0	20:1	15	60	25:1	12.88	13.36

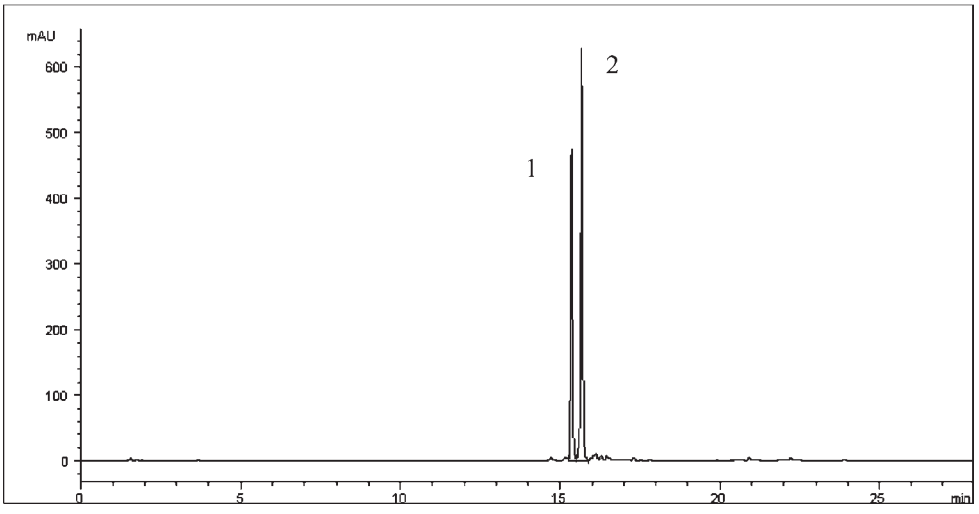

Figure 2. HPLC chromatogram of MCAE extract from *Camellia oleifera* Abel. meal. Peaks: 1, kaempferol-3-*O*-[2-*O*-*β*-D-galactopyranosyl-6-*O*-α-L-rhamnopyranosyl]-*β*-D-glucopyrnoside (KG1); 2, kaempferol-3-*O*-[2-*O*-*β*-D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl]-*β*-D-glucopyranoside (KG2).

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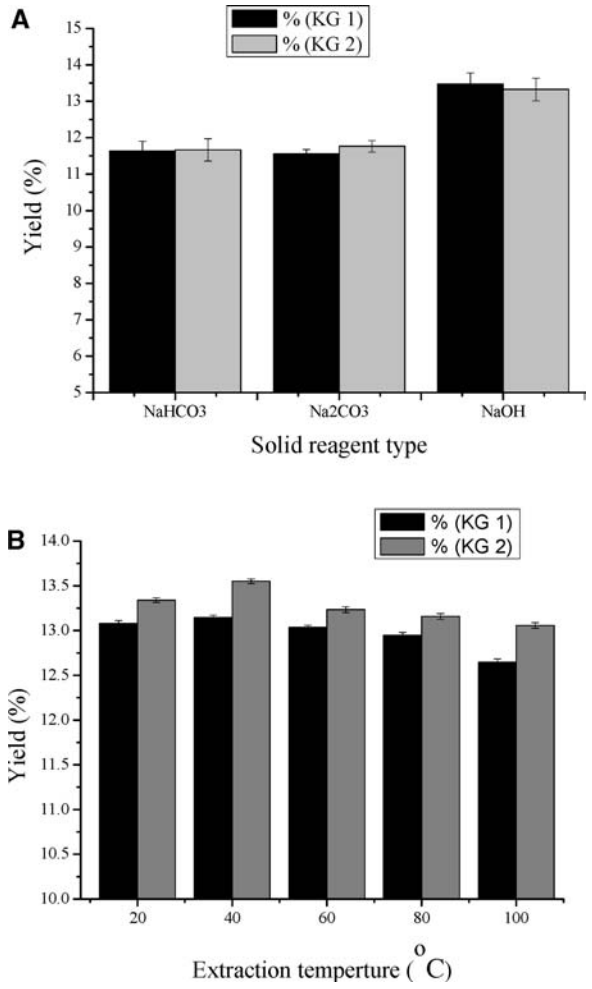

Figure 3. Effect of solid reagent type (A) and solid extraction temperature (B) on the yields of KG1 and KG2 in preliminary experiments.

Determination of Antioxidant Activity. The KGs obtained under optimal conditions were subjected to analysis of their antioxidant activity using a DPPH radical-scavenging assay and FTC.[27] All data were averages (± standard deviations) of triplicate determinations of three independent tests.

DPPH Radical-Scavenging Assay. The effect on DPPH radicals was determined according to the method of Duh et al.,[28] with slight modification. One milliliter of sample solution at various concentrations (20−120 μg/mL) was mixed with 4 mL of an alcoholic solution of DPPH (6.5×10^{-4} M). The reaction mixture was shaken vigorously and incubated for 20 min at room temperature, and then the absorbance was measured at 517 nm. Ascorbic acid (V_C) and BHT were used as reference compounds in the same concentration range as the test compounds. The hydroxyl radical scavenging activity (SA, %) was calculated by measuring the absorbance of the sample and applying the equation

$$SA(\%) = [1 - (A_i - A_j)/A_0] \times 100\% \quad (4)$$

where A_i is the absorbance of the reaction mixture, A_j is the absorbance of the reaction mixture without DPPH, and A_0 is the absorbance of the reaction mixture without KG.

FTC Assay. According to the mechanisms of this assay,[29] the antioxidant capacity was performed according to the thiocyanate method (FTC) described by Larrauri et al.[30] with some modifications. Four milliliters of extract, BHT, or V_C (2.0 mg/mL), 8.0 mL of Na−Pi buffer (50 mM, pH 7.0), and 4.0 mL of water or absolute ethanol were kept for 1 h. Then 4 mL of linoleic acid solution (2.5%) was added and shaken until saturated with oxygen. The test vials were put into an oven (40 °C) and analyzed at 24 h intervals. A 0.1 mL sample of assay mixture was mixed with 9.7 mL of ethanol (75%), 0.1 mL of thiocyanate (300 g/L), and 20 mM freshly prepared ferrous chloride solution (in 3.5% HCl). After 3 min of reaction, the absorbance was measured at 500 nm.

Table 3. Results of the Variance Analysis of Regression Model for the Extraction Yield of KG1

source	df	sum of squares	mean square	*F* value	Prob > F^a
model	14	45.48	3.25	33.67	<0.0001**
A	1	0.87	0.87	9.02	0.0095*
B	1	15.86	15.86	164.39	<0.0001**
C	1	7.004E−003	7.004E−003	0.073	0.7915
D	1	0.85	0.85	8.78	0.0103*
AB	1	0.15	0.15	1.60	0.2270
AC	1	0.15	0.15	1.52	0.2384
AD	1	2.756E−003	2.756E−003	0.029	0.8682
BC	1	0.15	0.15	1.60	0.2270
BD	1	6.250E−006	6.250E−006	6.478E−005	0.9937
CD	1	0.12	0.12	1.25	0.2821
A^2	1	5.91	5.91	61.28	<0.0001**
B^2	1	22.18	22.18	229.91	<0.0001**
C^2	1	4.94	4.94	51.17	<0.0001**
D^2	1	0.44	0.44	4.57	0.0507
residual	14	1.35	0.096		
lack of fit	10	1.21	0.12	3.45	0.1223
pure error	4	0.14	0.035		
cor total	29	46.84			

[a] *, $p < 0.05$, significant; **, $p < 0.01$, highly significant.

Table 4. Results of the Variance Analysis of Regression Model for the Extraction Yield of KG 2

source	df	sum of squares	mean square	*F* value	Prob > F^a
model	14	26.15	1.87	24.90	<0.0001**
A	1	1.40	1.40	18.68	0.0007
B	1	7.33	7.33	97.65	<0.0001**
C	1	0.024	0.024	0.32	0.5801
D	1	0.90	0.90	11.96	0.0038
AB	1	0.24	0.24	3.14	0.0984
AC	1	2.250E−004	2.250E−004	2.999E−003	0.9571
AD	1	1.000E−004	1.000E−004	1.333E−003	0.9714
BC	1	0.016	0.016	0.21	0.6551
BD	1	0.023	0.023	0.30	0.5926
CD	1	6.400E−003	6.400E−003	0.085	0.7745
A^2	1	1.03	1.03	13.75	0.0023
B^2	1	15.33	15.33	204.39	<0.0001**
C^2	1	2.02	2.02	26.94	0.0001
D^2	1	0.45	0.45	5.96	0.0285
residual	14	1.05	0.075		
lack of fit	10	0.86	0.086	1.77	0.3064
pure error	4	0.19	0.048		
cor total	29	27.35			

[a] *, $p < 0.05$, significant; **, $p < 0.01$, highly significant.

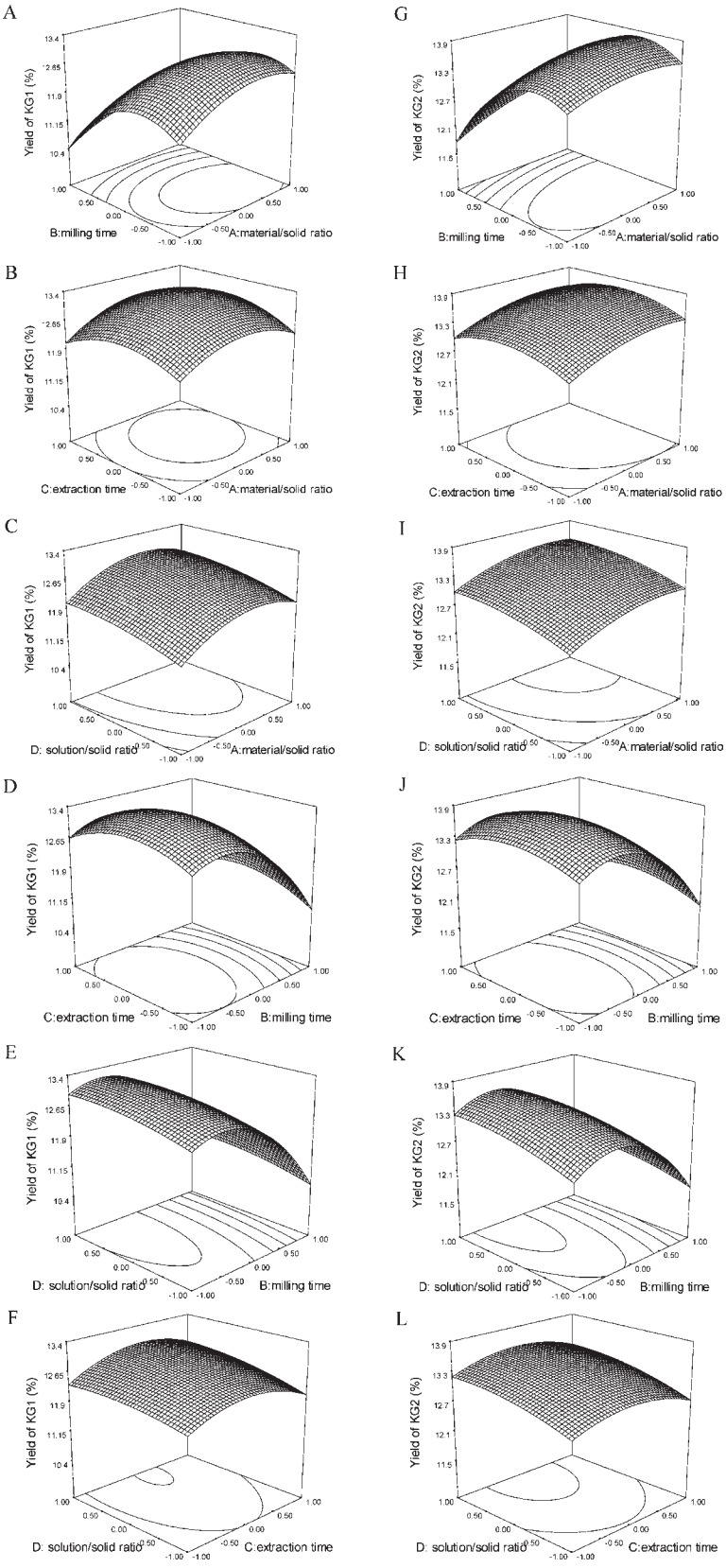

Figure 4. Response surface representations for KG1 (A−F) and KG2 (G−L): (A, G) various ratios of material to solid reagent and milling time; (B, H) various ratios of material to solid reagent and extraction time; (C, I) various ratios of material to solid reagent and ratio of solution to solid; (D, J) various milling times and extraction times; (E, K) various milling times and ratios of solution to solid; (F, L) various extraction times and ratios of solution to solid.

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RESULTS AND DISCUSSION

Preliminary Results. To determine the main factors and the appropriate ranges for the CCD, preliminary experiments were performed. There are many factors affecting the extraction efficiency of MCAE, such as solid reagent type and its amount, milling time, extraction time, ratio of solution to solid, and extraction temperature. However, among the variables screened, the ratio of material to solid reagent, milling time, extraction time, and ratio of solution to solid were identified as the most significant variables with ranges from 10:1 to 30:1 (g/g), 5 to 25 (min), 30 to 90 (min), and 15:1 to 35:1 (mL/g), respectively. The changes in extraction temperature with a range of 20−100 (°C) did not substantially influence the yields of KG1 and KG2 obtained by MCAE technique.

The effect of solid reagent type on the extraction yields was studied at the following conditions: ratio of material to solid reagent, 10:1 (g/g); milling time, 5 min; extraction time, 60 min; extraction temperature, 25 °C; and ratio of solution to solid, 20:1 (mL/g). As seen from Figure 3, when milling is performed with NaOH, the yield is higher than that with Na_2CO_3 or $NaHCO_3$. A possible reason is that the KG with low acid could not be neutralized completely by Na_2CO_3 or $NaHCO_3$. Hence, NaOH was selected as the optimal solid reagent.

On the basis of optimal solid reagent type, the effect of extraction temperature on the yields of KG1 and KG2 was investigated using temperatures of 20, 40, 60, 80, and 100 °C at a ratio of material to solid reagent of 20:1 (g/g), a milling time of 15 min, an extraction time of 60 min, and a ratio of solution to solid of 25:1 (mL/g) (Figure 3). As the extraction temperature was raised from room temperature to 40 °C, it was found that the yields of KG1 and KG2 were in an ideal range. When the temperature increased, the yields decreased significantly. On the basis of the tests, room temperature was a suitable condition for the experiment, which could save more energy than the traditional method.

Table 5. Comparison of MCAE with HRE

extraction method	extraction time	solvent	yield of KG1 (%)	yield of KG2 (%)
MCAE	60 min	water	13.14 ± 0.17	13.57 ± 0.19
HRE	3 h + 3 h	ethanol	12.56 ± 0.12	13.03 ± 0.15

Optimization of MCAE Operating Parameters. Further optimization of MCAE conditions was achieved by employing CCD. Data were analyzed using Design Expert 7.1.6 software for statistical analysis of variance (ANOVA), regression coefficients, and regression equation. The polynomial equations, describing the yields of KG1 (Y_1) and KG2 (Y_2) as a simultaneous function of amount of ratio of material to solid reagent (X_1), milling time (X_2), extraction time (X_3), and ratio of solution to solid (X_4), are shown in eqs 5 and 6, respectively.

$$Y_1 = 13.13 + 0.19X_1 - 0.81X_2 - 0.017X_3 + 0.19X_4 - 0.098X_1X_2 + 0.096X_1X_3 + 0.013X_1X_4 + 0.098X_2X_3 + 6.250\text{E} - 004X_2X_4 + 0.087X_3X_4 - 0.46X_1^2 - 0.90X_2^2 - 0.42X_3^2 - 0.13X_4^2 \quad (5)$$

$$Y_2 = 13.59 + 0.24X_1 - 0.55X_2 - 0.032X_3 + 0.19X_4 + 0.12X_1X_2 + 3.750\text{E} - 003X_1X_3 + 2.500\text{E} - 003X_1X_4 + 0.031X_2X_3 - 0.038X_2X_4 + 0.020X_3X_4 - 0.19X_1^2 - 0.75X_2^2 - 0.27X_3^2 - 0.13X_4^2 \quad (6)$$

To evaluate the optimal conditions of MCAE for KG1 and KG2 and the relationship between the response and the significant variables, ANOVA was performed. As shown in Tables 3 and 4, the experimental data fitted well to the quadratic models by ANOVA. The ANOVA for the response surface quadratic regression model showed that the model was highly significant ($P < 0.0001$) with high F values (33.67 for KG1 and 24.90 for KG2). The regression analysis of the data showed coefficient of determination (R^2) values for KG1 and KG2 of 0.9712 and 0.9614, respectively, which showed that the two models were significant. The adjusted determination coefficients (Adj R^2 = 0.9423 and for KG1 and Adj R^2 = 0.9228 for KG2) were also satisfactory to confirm the significance of the models. The lack-of-fit statistics, which was used to test the adequacy of the model, indicated that the P values for KG1 and KG2 (0.1223 and 0.3064) were not significant. No abnormality was obtained from the diagnoses of residuals. Thus, it can be concluded that the model was statistically sound.

Three-dimensional (3D) plots were highly recommended for the graphical interpretation of the interaction effect of independent variables on the response variables.[31] The effects of the independent variables and their mutual interaction on the yields of KG1 and KG2 can be seen on 3D response surface curves, and

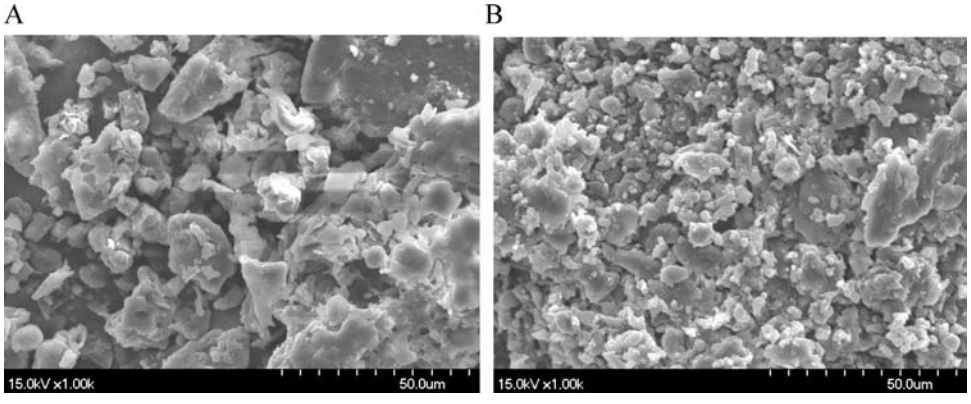

Figure 5. SEM micrographs of *Camellia oleifera* Abel. meal raw material (A) and milled with NaOH for 15 min (B).

contour plots are shown in Figure 4 (panels A−F and G-L). By solving the inverse matrix, the optimal parameters of the ratio of material to solid reagent of 21.75:1 (g/g), milling time of 12.95 min, extraction time of 60.9 min, and ratio of solution to solid of 29.05:1 (mL/g) were obtained on the basis of response surface.

Verification Experiments. The optimal conditions for MCAE were determined by Design Expert software. Under these optimum conditions, the verification experiments were conducted, and the observed values of the yields of KG1 and KG2 were 13.14 ± 0.17 and 13.57 ± 0.19%, respectively, which were not significantly different from the predicted values of 13.42 and 13.82%. The good correlation between these results confirmed that the response model was adequate to reflect the expected optimization. In view of the operating convenience, the optimal extraction parameters were found to be a ratio of material to solid reagent of 22:1 (g/g), milling time of 13 min, extraction time of 60 min, and ratio of solution to solid of 29:1 (mL/g). At the same time, the rationality and practicality of optimal conditions for MCAE were further confirmed in this study.

Comparison of MCAE with HRE. MCAE and HRE were compared for their performances of extracting KG1 and KG2 from *C. oleifera* Abel. meal at the optimized conditions. The extraction conditions and yields are listed in Table 5. The yields of KG1 and KG2 using MCAE were 4.6 and 4.1% higher than those using HRE, respectively. The extraction time of MCAE was only one-sixth of HRE. What is more, the solvent of the MCAE method was water, which was much safer and greener than the use of ethanol as the solvent of HRE. From the results, it is worth noting that application of MCAE offers a good alternative, environmentally friendly, and simplified route for active compound production.

SEM Observation. The impact-shift action on particles of a worked stock during mechanical activation is accompanied not only by grinding but also by destruction of cell shells. *C. oleifera* Abel. meal samples were examined by SEM to elucidate the morphological changes of samples using different extraction methods, which is helpful in understanding the extraction mechanism. Figure 5A shows a micrograph of raw *C. oleifera* Abel. meal powder, which has a majority of closed cells and highly rough surfaces. Figure 5B shows a detailed image of *C. oleifera* Abel. meal milled with NaOH for 15 min. The particle size of most of the obtained powder is obviously reduced, and the cell wall of *C. oleifera* Abel. meal was almost completely destroyed. The cell wall is intensively wrung and finally broken by the strong squeezing force and the shearing force through mechanical treatment, so the contents of cells are easily released and dispersed and chemical substances within the cell are rapidly released into the surrounding solvents.

Antioxidant Activity of KG. The antioxidant activity of KG prepared by MCAE and HRE was tested by two complementary test systems, namely, the DPPH radical-scavenging assay and the FTC assay, with the references of ascorbic acid and BHT. The samples were assayed over a range of dilutions, and the results of the DPPH radical-scavenging assay are shown in Figure 6. The concentration of sample producing a 50% reduction of the radical absorbance (IC_{50}) was used as an index to compare the antioxidant activity in the range of 0.02−0.12 mg/mL, and the radical-scavenging activity of KG increased along with sample concentration. Compared with the KG obtained by HRE (IC_{50} = 0.1012 mg/mL), KG obtained by MCAE exhibited better hydroxyl radical scavenging activity with an IC_{50} value of 0.0850 mg/mL.

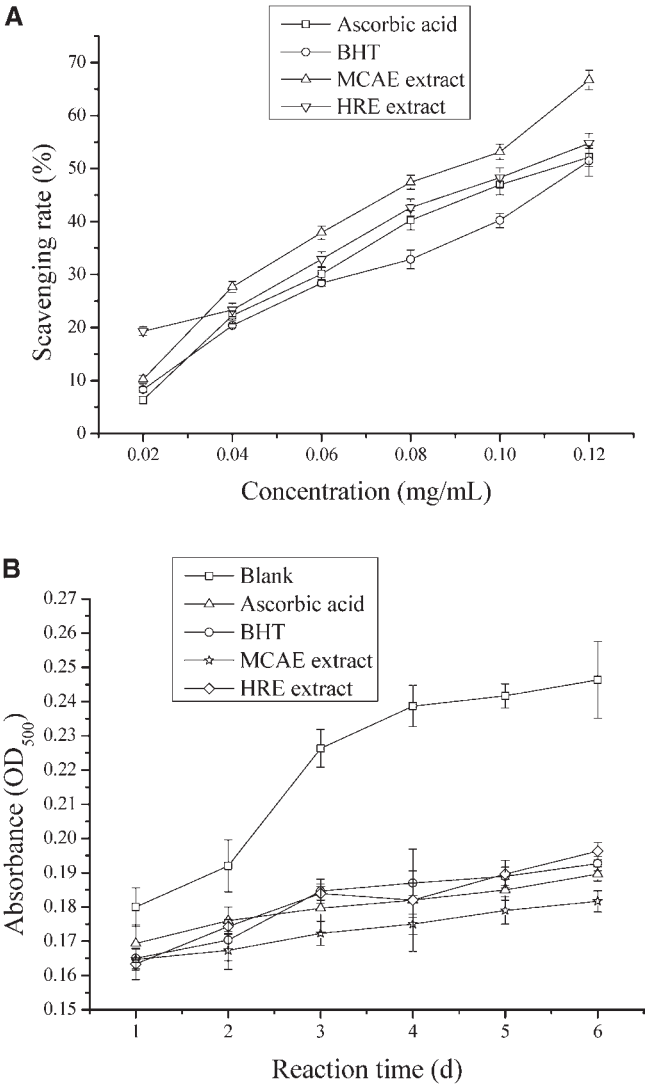

Figure 6. (A) Free radical-scavenging activity of MCAR extract, HRE extract, ascorbic acid, and BHT. (B) Antioxidant activities of control, ascorbic acid, BHT, MCAE extract, and HRE extract as assessed by inhibiting peroxidation of linoleic acid method.

To further confirm the antioxidant activity of KG extracted by MCAE and HRE, the FTC assay was performed, and it was apparent that KG obtained from MCAE showed a good inhibition of linoleic acid peroxidation as compared with KG obtained by HRE, blank sample, BHT, and ascorbic acid (Figure 6). The absorbance of the HRE extracts increased from 0.163 to 0.196, whereas the MCAE extracts had lower rates maintaining 0.165 and 0.182, respectively.

In conclusion, through RSM, the optimization of a MCAE procedure to maximize the kaempferol glycosides yields from *C. oleifera* Abel. meal was achieved. The optimized conditions allow for 4.6 and 4.1% higher yields of KG1 and KG2 than those using HRE, respectively. The antioxidant activities of the extracts have been evaluated by DPPH radical-scavenging assay and FTC assay. On the basis of the results, MCAE, which has notable advantages of reducing organic solvent, saving time, lower temperature, and higher efficiency, represents a valuable alternative to the traditional HRE for the efficient extraction of KG from *C. oleifera* Abel. meal.

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