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Microwave-assisted extraction of ginsenosides from ginseng root

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Abstract

The extractions of ginsenosides Rg_1 and Rb_1 from ginseng root under atmospheric pressure by focused microwave-assisted technique have been investigated. The parameters used for the optimization were solvent composition, extraction time, and applied microwave power. The ginsenosides were quantified by high-performance liquid chromatography equipped with UV/Vis detector. The results of the 15-min microwave-assisted extraction (0.28% of Rg_1 obtained in 70% water–ethanol and 1.31% of Rb_1 obtained in 30% water–ethanol under 150 W of microwave power) were better than that from 10-h conventional solvent extraction (0.22% of Rg_1 and 0.87% of Rb_1 obtained in 70% water–ethanol).

Keywords: *Panax ginseng*; Ginsenosides; Microwave; Extraction

1. Introduction

Ginseng, the root of *Panax ginseng*, has been used for thousands of years as a traditional medicine in many oriental countries processing central nervous system-stimulating, cardiotonic and hypotensive effects [1]. It has also gained popularity in the western world during the last couple of decades and evidence was given that ginseng may improve the quality of life in a middle-aged population [2]. The active constituents are ginsenosides that include neutral ginsenosides, malonylginsenosides, and oleanolic acid-type ginsenoside [3–5]. More than 30 ginsenosides are known, the most abundant components present in ginseng are Rb_1, Rb_2, Rc, Rd, m-Rb_1, m-Rb_2 and m-Rc, which consist of 20(S)-protopanaxadiol aglycon moiety; and Re, Rf, Rg_1 and Rg_2, which possess 20(S)-protopanaxatriol as an aglycon. Ginsenosides are frequently used as main index for ginseng product evaluation. High-performance liquid chromatography (HPLC) methods (either in normal-phase or reverse-phase mode) with ultraviolet, near-infrared reflectance, or mass spectrometric detection [6–8] and gas chromatography methods with mass spectrometric detection [9] methods have been developed for simultaneous analysis of the main ginsenosides.

Microwave-assisted extraction (MAE) has been shown to enhance the extraction efficiency of interested components from a wide variety of sample matrices and has been used as an alternative sample preparation technique for a number of applications [10–14]. This method has been

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employed for the extraction of pollutants such as polycyclic aromatic hydrocarbons [15,16], hydrocarbons [17], organochlorine pesticides [18], polychlorinated biphenyls [19], dioxins/furans [20,21], triazines [22] and alkyl/aryl phosphates [23], from environmental matrices and natural products such as essential oils from plant materials [24,25], glycyrrhizic from licorice root [26], taxans from *Taxus* biomass [27], and azadirachtin-related limonoids from neem [28]. Under an optimized MAE conditions, with extraction times of 4–5 min in 100 ml of 50–60% ethanolic aqueous solution containing 1–2% (v/v) ammonia and 10:1 liquid–solid ratio, the recovery of glycyrrhizic acid from licorice root was equivalent to conventional extraction methods such as Soxhlet (10 h in 200 ml solution), reflux (4.5 h with 260 ml solution) and ultrasonic (30 min after 20 h room temperature extraction in 100 ml solution) methods [26]. Parameters such as temperatures, times, biomass to solvent ratios and solvents for the MAE procedure were also investigated to optimize the efficiency of the extraction of taxanes from *Taxus* needles. With optimized parameter settings, MAE was found considerably to reduce both extraction time (from 16 h to 9:10 min) and solvent consumption (from 100 to 20 ml with methanol) while comparing with overnight conventional shaking method [27]. In the extraction of azadirachtin from seed shell, leaf, and stem of neem tree by MAE, the operating parameter such as solvent, irradiation time, and microwave power have an effluence on the efficiency for the extractions. Significant enhancement of extraction efficiency of azadirachtin-related limonds over traditional room temperature extraction and reflux extraction methods was observed [28]. Conditions for MAE of tanshinone I, tanshinone IIA and cryptotanshinone from the root of *Salvia miltiorrhiza bunge* were also reported [29]. The percentage extraction by MAE was the same or even better than conventional methods. MAE only takes 2 min, but room temperature, reflux, ultrasonic and Soxhlet extractions need 24 h, 45 min, 75 min and 90 min, respectively. The advantages observed from the MAE are to reduce the volume of solvents required, enhance extraction efficiency, reduce extraction time, and improve the precision of analyte recoveries and to decrease the costs.

Extraction of ginseng by conventional heating in ethanolic aqueous solvent is a prolonged process [30,31]. The aim of this work is to compare the extraction efficiencies of ginsenosides Rg_1 and Rb_1 (Fig. 1) from ginseng by microwave and conventional solvent extraction. The parameters studied, which might affect the extraction efficiency, were extraction time, solvent composition and microwave power. This information might be essential for the production of better ginseng extracts.

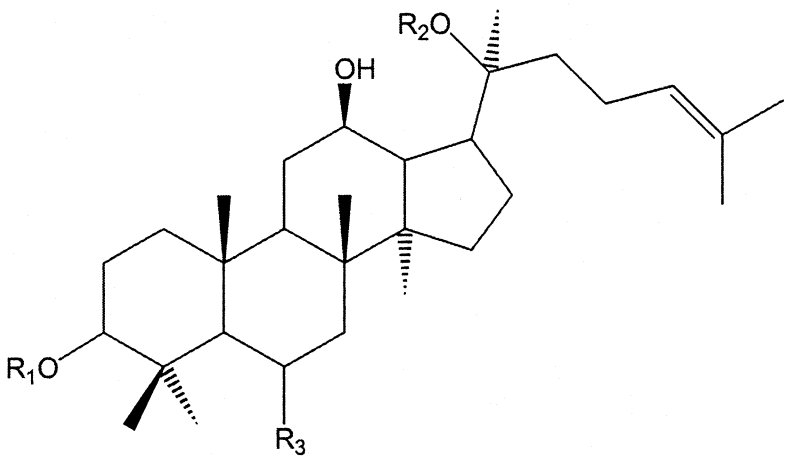
2. Experimental

2.1. Materials

Ginsenosides (Rg_1 and Rb_1) and 6-year-old Korean ginseng roots (moisture content: 7.8%) were obtained from School of Pharmacy, China Medical College in Taichung, Taiwan. The ginseng roots were ground with a blender and screened to 80 mesh. Solvents used in this study were acetonitrile (HPLC grade, Fisher Scientific, USA), methanol (HPLC grade), water (HPLC Grade, Houfong, Taiwan) and ethanol (95%, Taiwan Tobacco and Wine Co.). Nylon syringe filters (13 mm i.d., 0.2 μm) were purchased from Supelco (US).

2.2. Microwave-assisted extraction

A Prolabo microwave facility (Soxwave 100, 2450 MHz) equipped with a programmable heating power from 0 to 300 W with 5% increment was used. Sample was weighed exactly (~2.0 g) and placed in a 250 ml quartz extraction cell equipped with reflux system. All the microwave extractions were performed under a set microwave irradiation power (30 and 150 W) for a certain period of time (1, 2, 5, 10 and 15 min) in 15.0 ml of solvent (A: 100% H_2O, B: 70% H_2O/ethanol, C: 50% H_2O/ethanol, D: 30% H_2O/ethanol, and E: 5% H_2O/ethanol). The extracts were filtered through a syringe filter for subsequent HPLC–UV determination. Ethanol has higher dissipation factor at 2450 MHz of microwave frequency and volatile



	R_1	R_2	R_3
Rb_1	D-Glc(β1-2)D-Glc-	D-Glc(β1-6)D-Glc-	H-
Rg_1	H-	D-Glc-	D-Glc-O-

Glc: glucose

Fig. 1. Structures of ginsenosides.

character than water, therefore, safety guidelines regarding work with microwave field should be observed.

2.3. Conventional solvent extraction

Solvent extractions were performed between 80 and 100 °C at azeotropic temperature of the solvents in a water or oil bath with reflux apparatus using the same amount of sample and solvent as in the microwave extraction. The extracts were then filtered through syringe filter.

2.4. High-performance liquid chromatography determination

HPLC was conducted on a Hewlett Packard HP 1100 liquid chromatograph equipped with binary pump G1312A and variable wavelength detector G1314A. Ten microliter of the ginseng extracted was injected and analyzed by using a Hypersil ODS (250×4.0 mm^2, 5 μm) at room temperature. The mobile phase consisted of solvent I (acetonitrile) and solvent II (water). A gradient procedure was used: 0–20 min, 20–22% I, 80–78% II; 20–45 min, 22–46% I, 78–54% II; 45–50 min, 46–55% I, 54–45% II; 50–55 min, 55–100% I, 45–0% II; 55–60 min, 100–20% I, 0–80% II. The flow rate was kept at 1.0 ml/min and the absorbance was measured at a wavelength of 203 nm for the detection of ginsenosides. The chromatograms are illustrated in Fig. 2.

The linear dynamic range was established by a five-point calibration curve (e.g. 500. 250, 50, 10 and 5 ng/μl) with correlation coefficients of 0.999 and 0.998 for Rg_1 and Rb_1, respectively. The detection limit were 0.02 μg for both ginsenosides.

3. Results and discussion

The temperature during microwave heating was difficult to control when the extractions occur at a temperature below the solvent boiling point. In this experiment, the sample solution was heated at

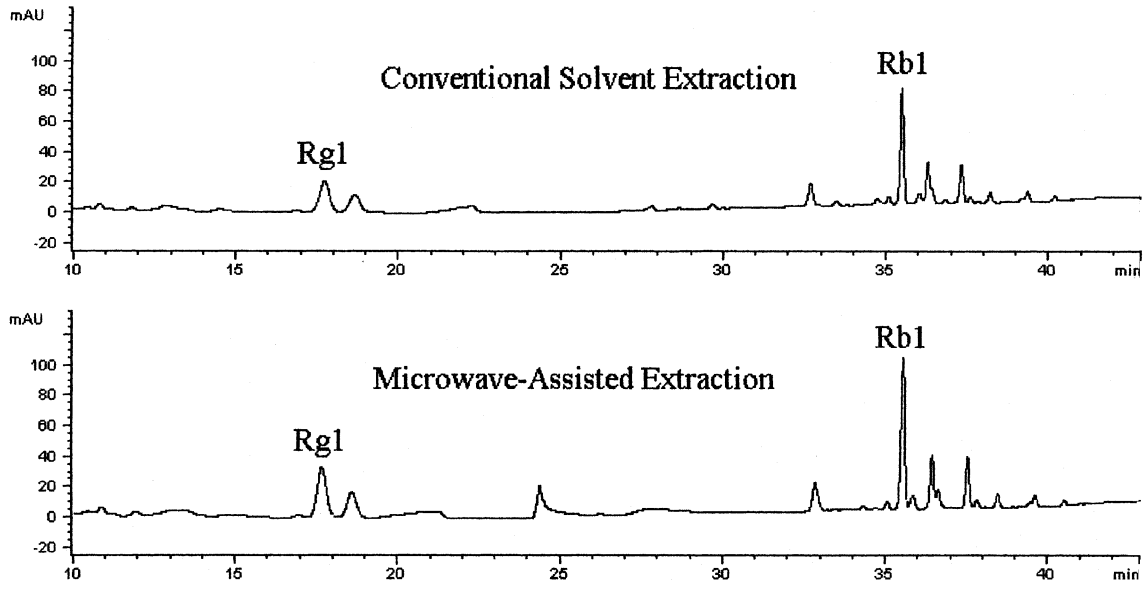


Fig. 2. High-performance liquid chromatogram of ginsenosides Rg_1 and Rb_1.

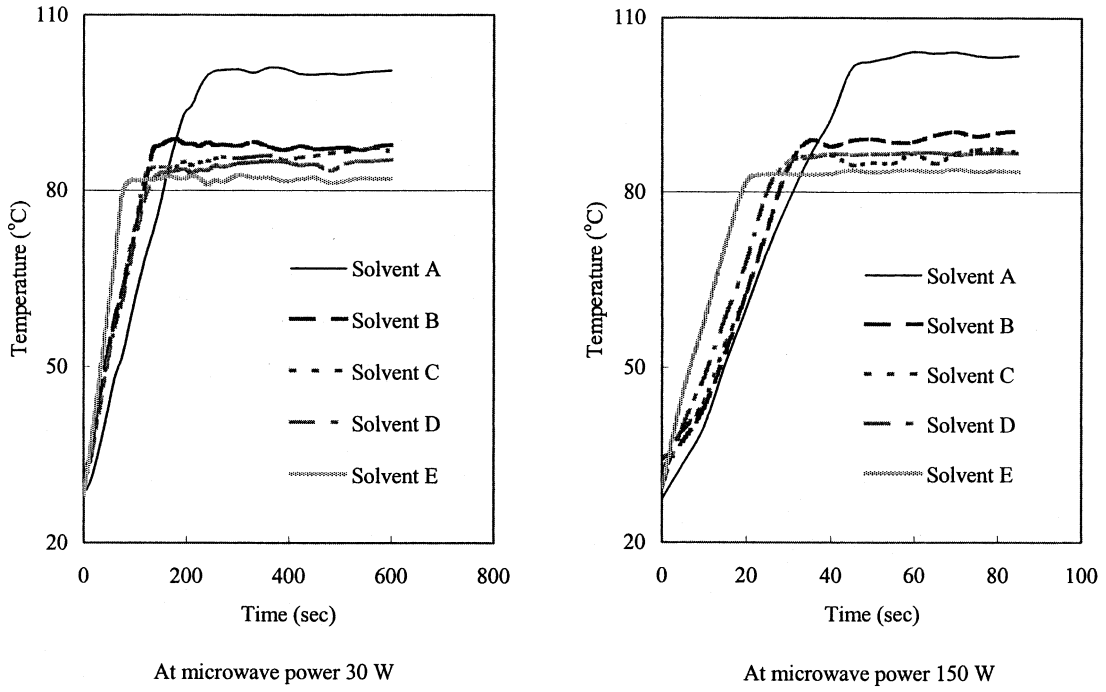


Fig. 3. Temperature profiles of microwave heating.

Table 1
Results of ginsenosides Rg_1 and Rb_1 extracted by microwave-assisted method

Solvent	Time (min)	Milligram of ginsenosides extracted from 1 g of ginseng (average ± S.D.)[a]					
		Rg_1 (30 W)	Rb_1 (30 W)	Rg_1/Rb_1	Rg_1 (150 W)	Rb_1 (150 W)	Rg_1/Rb_1
A	1	0.38 ± 0.08	1.37 ± 0.18	0.28	1.23 ± 0.15	4.79 ± 0.26	0.26
	2	0.88 ± 0.09	2.28 ± 0.21	0.38	1.64 ± 0.21	5.75 ± 0.17	0.29
	5	1.46 ± 0.16	5.09 ± 0.17	0.29	2.14 ± 0.08	6.42 ± 0.19	0.33
	10	2.26 ± 0.07	6.92 ± 0.36	0.33	2.50 ± 0.11	6.97 ± 0.21	0.36
	15	2.76 ± 0.09	8.38 ± 0.28	0.33	2.76 ± 0.13	7.13 ± 0.25	0.39
B	1	0.43 ± 0.05	1.61 ± 0.35	0.27	0.86 ± 0.13	2.78 ± 0.26	0.31
	2	0.87 ± 0.07	3.07 ± 0.24	0.28	1.51 ± 0.26	4.78 ± 0.15	0.32
	5	1.42 ± 0.08	4.94 ± 0.24	0.29	2.05 ± 0.08	6.72 ± 0.28	0.31
	10	1.93 ± 0.10	6.69 ± 0.31	0.29	2.53 ± 0.09	8.08 ± 0.39	0.31
	15	2.39 ± 0.08	8.17 ± 0.56	0.29	2.83 ± 0.12	9.03 ± 0.36	0.32
C	1	0.23 ± 0.05	0.91 ± 0.09	0.25	0.78 ± 0.11	2.78 ± 0.29	0.28
	2	0.48 ± 0.04	1.73 ± 0.13	0.28	1.42 ± 0.14	5.21 ± 0.35	0.27
	5	1.18 ± 0.13	3.54 ± 0.19	0.33	1.86 ± 0.12	6.70 ± 0.51	0.28
	10	1.69 ± 0.07	5.02 ± 0.16	0.34	2.13 ± 0.13	7.49 ± 0.41	0.28
	15	2.02 ± 0.08	5.82 ± 0.15	0.35	2.39 ± 0.15	8.05 ± 0.55	0.30
D	1	0.32 ± 0.05	1.38 ± 0.14	0.23	1.13 ± 0.13	5.45 ± 0.34	0.21
	2	0.73 ± 0.07	2.88 ± 0.18	0.25	1.78 ± 0.15	8.47 ± 0.25	0.21
	5	1.24 ± 0.06	5.35 ± 0.15	0.23	2.13 ± 0.13	9.82 ± 0.26	0.22
	10	1.77 ± 0.14	7.60 ± 0.13	0.23	2.59 ± 0.23	12.10 ± 0.33	0.21
	15	2.19 ± 0.10	8.96 ± 0.31	0.24	2.83 ± 0.22	13.07 ± 0.49	0.22
E	1	0.18 ± 0.06	1.73 ± 0.26	0.10	0.26 ± 0.06	3.15 ± 0.27	0.08
	2	0.43 ± 0.07	2.74 ± 0.16	0.16	0.52 ± 0.06	5.98 ± 0.17	0.09
	5	0.82 ± 0.08	3.76 ± 0.13	0.22	0.73 ± 0.09	8.86 ± 0.75	0.08
	10	1.11 ± 0.12	6.65 ± 0.85	0.17	1.14 ± 0.14	10.46 ± 0.68	0.11
	15	1.25 ± 0.15	9.12 ± 0.39	0.14	1.32 ± 0.12	11.73 ± 0.53	0.11

[a] Triplicate extraction.

a set microwave output level for a certain period of time during the extraction. The boiling points of the extraction solvents vary with their compositions, and the time needed to reach their boiling point are also different (Fig. 3). Therefore, microwave power and extraction time were taken into consideration as parameters that might affect the extraction efficiency.

3.1. Microwave power effect

Amount of ginsenosides extracted by MAE method under different microwave conditions are represented in Table 1 and Fig. 4. In general, the extraction efficiency was improved by raising microwave power from 30 to 150 W. During short extraction time (1 and 2 min), both recoveries of Rg_1 and Rb_1 were enhanced with increased microwave power. When the extraction solutions were heated long enough (15 min), the yields of 150 W were higher than that of 30 W for most of the cases except for the extraction carried out in solvent A. The difference of the ginsenosides extracted between 30 and 150 W appears to be more significant with short extraction times compared to long extraction times. The temperature profile of microwave heating (Fig. 3) shows that the time required to reach boiling points (or azeotropic point) of the solutions at 150 W is approximately one-fifth of the time required at 30 W.

3.2. Extraction time

As in other extraction techniques, time is one of the parameters whose influence needs to be

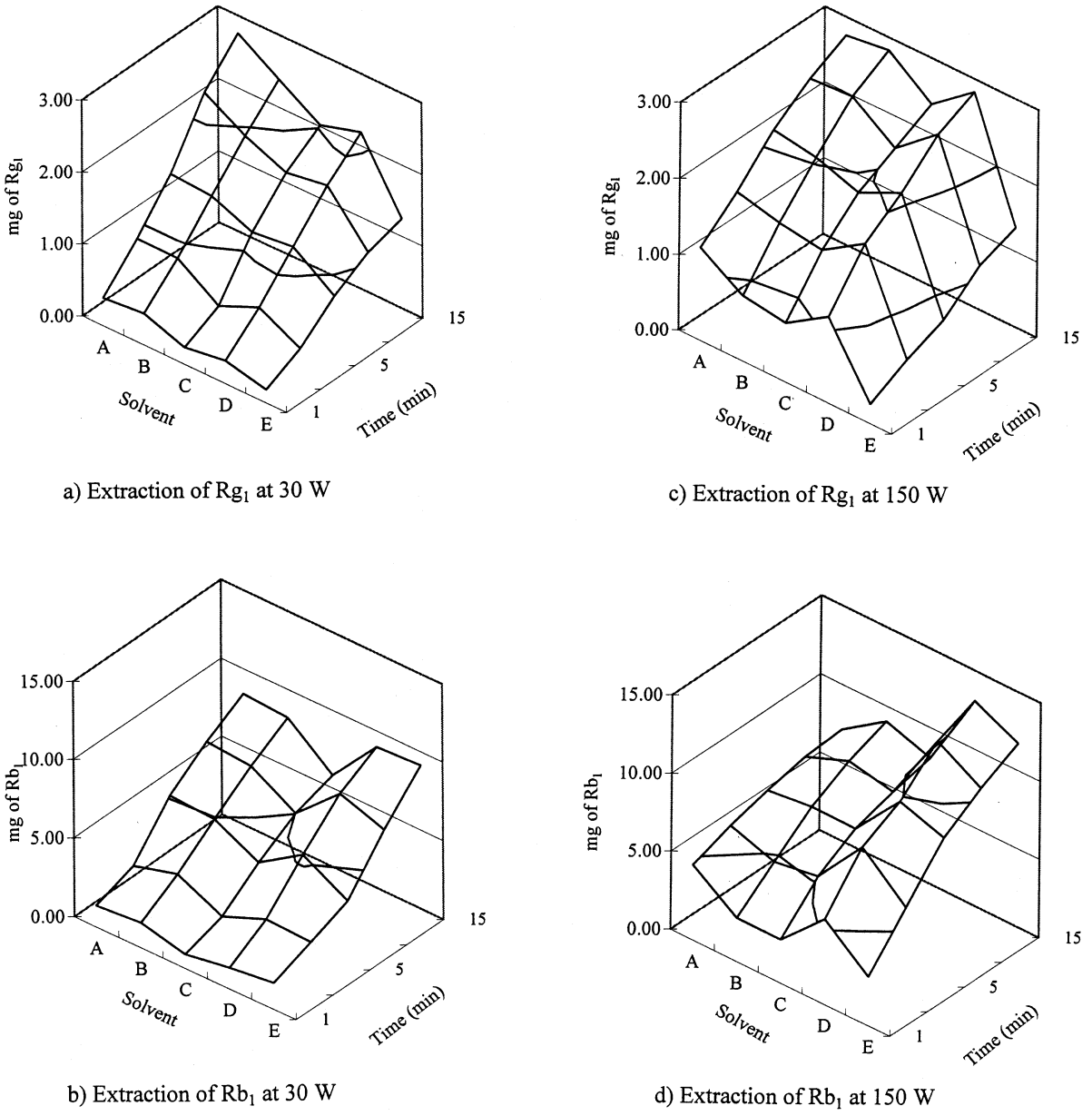


Fig. 4. Amount of ginsenosides extracted as a function of time and solvent by MAE.

taken into account. As an example, increasing the extraction time resulted in a higher percentage of glycyrrhizic acid extracted form licorice root [22]. However, longer extraction times may not have further effects or negative effects resulting from degradation or conversion of the analytes. Within these present MAE results, in general, the longer the extraction time, the greater the recovery of the ginsenosides extracted. It is observed that the 15-min extraction gave the highest yield in all solvent systems under 30 and 150 W of the microwave. With 30 W microwave irradiation, the highest

Table 2
Results of ginsenosides Rg_1 and Rb_1 extracted by conventional solvent extraction

Solvent	Time (min)	Milligram of ginsenosides extracted from 1 g of ginseng (average ± S.D.)[a]		
		Rg_1	Rb_1	Rg_1/Rb_1
A	10	0.34 ± 0.06	0.83 ± 0.13	0.41
	40	0.86 ± 0.12	2.33 ± 0.27	0.37
	220	1.38 ± 0.16	3.64 ± 0.25	0.38
	640	1.78 ± 0.12	4.64 ± 0.18	0.38
B	10	0.55 ± 0.16	1.77 ± 0.13	0.31
	40	1.13 ± 0.10	4.56 ± 0.27	0.25
	220	2.06 ± 0.19	8.24 ± 0.37	0.25
	640	2.21 ± 0.18	8.74 ± 0.28	0.25
C	10	1.01 ± 0.12	3.30 ± 0.29	0.31
	40	1.47 ± 0.11	5.51 ± 0.33	0.27
	220	1.92 ± 0.10	7.58 ± 0.61	0.24
	640	2.19 ± 0.18	8.63 ± 0.55	0.25
D	10	0.64 ± 0.10	2.72 ± 0.15	0.24
	40	1.17 ± 0.09	4.92 ± 0.31	0.24
	220	1.87 ± 0.18	7.74 ± 0.52	0.24
	640	2.18 ± 0.11	8.35 ± 0.39	0.26
E	10	0.44 ± 0.09	0.06 ± 0.03	[b]
	40	0.83 ± 0.11	0.88 ± 0.19	[b]
	220	1.48 ± 0.23	2.77 ± 0.31	0.53
	640	1.85 ± 0.11	3.48 ± 0.25	0.23

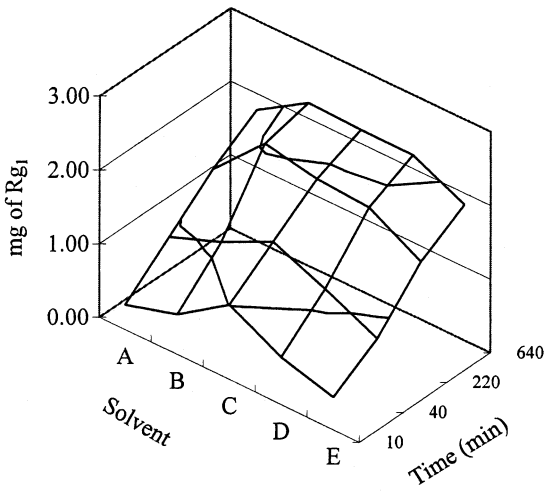
[a] Triplicate extraction.
[b] Ratio is not counted due to large S.D.

recovery of Rg_1 was obtained in solvent A (2.76 mg/g) and that for Rb_1 was obtained in solvent E (9.12 mg/g). With 150 W of microwave power, the best yield of Rg_1 was found in solvents B and C (2.83 mg/g) and that for Rb_1 was found in solvent D (13.07 mg/g).

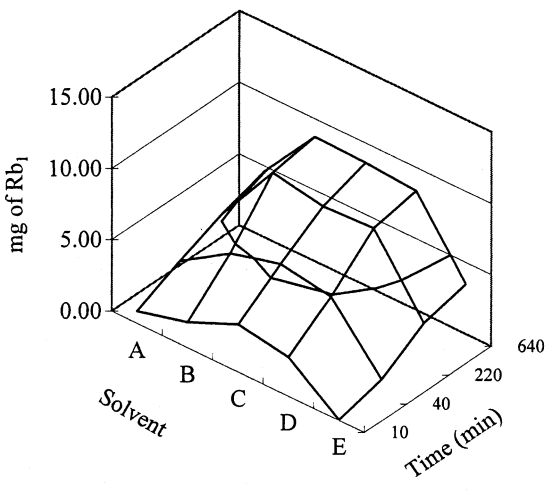
3.3. Solvent effect

Under the 15-min and 150-W microwave power extraction condition, the quantities of Rg_1 extracted in solvent A, B and D were similar and in a range between 0.276 and 0.283% (w/w). Slightly lower recovery (0.239%) was found in solvent C, and the lowest (0.132%) was observed in solvent E. As for the extraction of Rb_1, the highest extraction efficiency was performed in solvent D (1.31%) and the lowest was performed in solvent A (0.713%). The relative standard deviations of the triplicate analysis were in the range of 1.7–13.1% for most of the extraction conditions.

Results of conventional solvent extraction are given in Table 2. The profiles of extraction efficiency of Rg_1 and Rb_1 are similar (Fig. 5), with higher yields observed for solvents B, C and D. In general, the ginsenosides extracted after 10-h



a) Extraction of Rg_1 by conventional solvent heating



b) Extraction of Rb_1 by conventional solvent heating

Fig. 5. Amount of ginsenosides extracted as a function of time and solvent by conventional solvent extraction.

CSE were still lower than those extracted after only 15-min MAE.

To perform MAE with the same solvent as is prescribed for the traditional extraction is usually an approach to start with. The ability of material to absorb microwave energy is defined by its dissipation factor tan δ, which is the ratio of the dielectric loss (ε'') to dielectric constant (ε') of the material, when the former (ε'') is a measure of material's ability to absorb microwave energy and convert it into heat and/or other forms of energy. Therefore, the optimal extraction solvents for MAE cannot always be reasoned directly from those used in conventional methods.

The solvents behave differently in these two extraction techniques. Among all solvents used, extraction carried out in 95% ethanolic solution (solvent E) resulted in a significant lower recovery of Rg_1 as compared to the other solvents used (Fig. 4a–c). With the same solvent E in the CSE method, the amount of Rg_1 extracted was found only slightly lower (Fig. 5a) than the amount obtained using the other solvents. However, in the extraction of Rb_1 in solvent E, the following results were observed: using MAE at 150-W and 15-min extraction, the recovery of Rb_1 was slightly lower than that in solvent D but significantly higher than in solvents A, B and C (Fig. 4d); while in CSE, the recovery for solvent E was the lowest among all the solvents used (Fig. 5b). When the extractions were carried out in solvent C (50% ethanol–water) by MAE, the percentages of Rg_1 (0.239%) and Rb_1 (0.805%) were not the highest yields. However, the highest yields (Rg_1: 0.219%; Rb_1: 0.863%) were obtained by CSE when the extraction solvent C was used. Similarity of the HPLC–UV chromatograms of CSE and MAE were observed (Fig. 2). Rg_1, Rb_1 and other major peaks are correspondent between the two chromatograms. An unidentified peak appears at retention time of 24.5 min, which might be due to the extra component extracted by MAE observed under the chromatographic condition. ANOVA was performed at the 95% confidence level for the analysis of MAE results which indicated that significant differences were not observed: among solvent A, B and D in the extraction of Rg_1; and, between solvent A and B in the extraction of Rb_1.

The ratios of Rg_1 to Rb_1 under different extraction conditions ranged between 0.08–0.39 by MAE and 0.24–0.53 by CSE. Depending on the selection of solvent and extraction method, the composition of ginsenosides in ginseng extract may be varied.

Ren and Chen [7] reported the degradation rate of ginsenosides in ginseng extract during microwave and conventional heating were similar and concluded microwave energy may be applied to the extraction of ginsenoside and thermal sterilization of the ginseng products. In this present study, we found that the extraction of the two ginsenosides Rg_1 and Rb_1 by microwave energy were very efficient. The rate of the extraction by MAE is much faster than that by CSE. The advantage of the faster extraction may avoid some thermal degradation that might occur during a prolonged heating.

MAE has been shown to be an efficient method for extraction of ginsenosides Rg_1 and Rb_1 from ginseng root. Compared with the conventional heating method, the MAE procedure provided the enhanced extraction efficiency and the reduction of processing time. MAE is a feasible alternative method for the extraction of interested ingredients from some biological materials.

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