

Microwave Degradation of Floatation-Enriched Ginsenoside Extract from *Panax quinquefolium* L. Leaf

YUPING BAI, LISHA ZHAO, CHENLING QU, XIANGZHE MENG, AND HANQI ZHANG*

College of Chemistry, Jilin University, Changchun, 130012, P. R. China

Even though the degradation of ginsenosides has been thoroughly studied in animals and in vitro using acids, enzymes, and intestinal bacteria, a new degradation method is established for obtaining the ginsenosides Rg₃, Rh₂ and their aglycon 20(S)-protopanaxadiol. This method is based on the microwave irradiation degradation of the major ginsenosides from the *Panax quinquefolium* L. coupled with foam floatation. The results indicated that ginsenosides Rg₃, Rh₂ and aglycon are not naturally present in *Panax quinquefolium* L., but are the products obtained simultaneously in the microwave irradiation degradation process. The yield of Rg₃ is 7.69 mg/g and 250 times as high as that obtained from red ginseng, and the transformation rate of total ginsenosides to aglycon is 78.11%. It is important to stress that a new degradation medium, *N,N*-dimethylformamide, was discovered and, when the medium was used, the transformation rate of total ginsenosides to aglycon was 20.20%. The proposed method is simple, efficient, time saving and a noteworthy improvement on the traditional degradation methods such as acidic hydrolysis, alkaline degradation, enzymatic conversion or microbial degradation.

KEYWORDS: *Panax quinquefolium* L.; degradation; foam floatation; HPLC–APCI/MS; microwave irradiation; ginsenoside; anticancer activities

INTRODUCTION

Panax quinquefolium L. has been not only used as therapeutic agents but also marketed as dietary supplements and raw materials of healthy food. For example, the roots of *Panax quinquefolium* L. have been used as additives to drinks for hundreds of years. Ginsenosides, including neutral ginsenosides and malonyl ginsenosides, are known to be the main bioactive components of *Panax quinquefolium* L. (1, 2). Mostly derived from tetracyclic triterpene dammarane, ginsenosides are subdivided into protopanaxadiol, protopanaxatriol and oleanolic acid ginsenosides. The structures of main ginsenosides are shown in Figure 1.

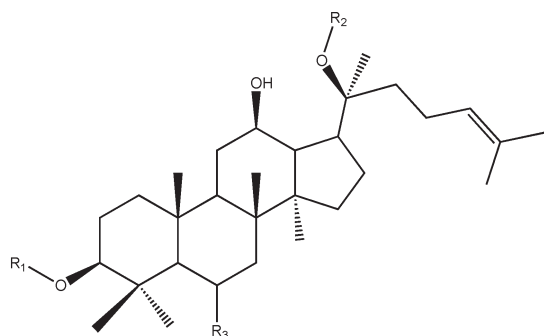
The root of *Panax quinquefolium* L. is a typical source for use in many traditional medicinal therapies. Compared with the root, the leaf was less investigated. Annual recovery of ginseng leaf could be a feasible alternative source of ginsenosides compared with the roots requiring a long growth cycle for nutritious products. It was found that the content of total ginsenosides in leaf is much higher than that in root (3). The leaf of *Panax quinquefolium* L. is a source of ginsenosides but has not been well exploited up to now (3, 4).

Rare ginsenosides, including Rg₂, Rg₃, Rg₅, Rh₁, Rh₂, Rh₃ and Rck, can be obtained from deglycosylation of the major ginsenosides. Compared with the major ginsenosides, rare ginsenosides have higher bioactivity than major ginsenosides and many research studies indicated that the metabolites of major ginsenosides

in vivo and in vitro which belong to rare ginsenosides are bioactive compounds (5–8). Among the rare ginsenosides, Rg₃ and Rh₂ are of greatest interest because their bioactivity is higher than others especially in the aspect of antitumor activity. It has been reported that Rh₂ can not only reduce the proliferation of a variety of cultured cancer cells but also influence apoptosis (9). In addition, Rg₃ has been shown to possess antitumor properties and influence drug resistant cultured cancer cells (10, 11). Bae et al. (12) reported that ginsenoside Rg₃ is metabolized to ginsenoside Rh₂ and 20(S)-protopanaxadiol when anaerobically incubated with human fecal microflora, and the activities of deglycosylated metabolites are comparable to or higher than that of Rg₃ (9, 13). Characteristics and mechanism of effects among the three have been proved similar, but the sequence of functional strength is 20(S)-protopanaxadiol, Rh₂ and Rg₃, which probably has a close relation with the fact that 20(S)-protopanaxadiol is the aglycon of Rg₃ and Rh₂ (14, 15). Thus, preparation and determination of Rg₃, Rh₂ and 20(S)-protopanaxadiol are significant for their biological and pharmacological activities.

The degradation of ginsenosides is to remove several or all the glycosyl moieties in order to obtain rare ginsenosides or the aglycon. Ginsenosides, especially malonyl ginsenosides, are thermally unstable, and they may degrade during thermal extraction (16). Thermal degradation of neutral ginsenosides in aqueous solution during conventional heating has been extensively studied (17–19). But no or just a small amount of rare ginsenosides were obtained. Many degradation methods for obtaining rare ginsenosides, including mild acid hydrolysis (20), alkaline degradation (21), enzymatic conversion (22) and microbial degradation (13), have been introduced extensively for a long time.

*Corresponding author. Mailing address: College of Chemistry, Jilin University, 2699 Qianjin Street, Changchun, 130012, P. R. China. E-mail: analchem@mail.jlu.edu.cn. Fax: +86-431-85112335. Tel: +86-431-85168399.



Ginsenoside	R ₁	R ₂	R ₃	Molecular weight
Rb ₂	Glc ² -Glc	Glc ² -Arap	H	1078
Rb ₃	Glc ² -Glc	Glc ⁶ -Xyl	H	1078
Rc	Glc ² -Glc	Glc ⁶ -Araf	H	1078
Rd	Glc ² -Glc	Glc	H	946
Rg ₃	Glc ² -Glc	H	H	784
Rh ₂	Glc	H	H	622
aglycone	H	H	H	460

Figure 1. Structures of main ginsenosides.

The degradation of ginsenosides in humans after oral administration has been also reported (23). Though there were some degradation methods to obtain rare ginsenosides, further efforts devoted to method development are required. For instance, side reactions, such as epimerization, hydration, cyclization and hydroxylation, often occur during mild acid hydrolysis degradation (20). Most of the micro-organisms used for the degradation of ginsenosides are not of a food-grade standard, and the enzymatic conversion degradation process is complex and of high cost (24). Because the alkaline degradation process is simple and the side reactions are little, it has been mostly studied recently. However, the yields of rare ginsenosides obtained by all the conventional degradation methods are very low and all the degradations are slow processes. Recently, microwave heating has been not only employed for the extraction of major ginsenosides, in which faster extraction rates were observed under certain conditions (25,26), but also applied to the degradation of malonyl ginsenosides (16). Therefore, it is likely that microwave heating may also enhance the degradation of neutral ginsenosides. Foam floatation has been recognized as a suitable technique for the separation and purification of surface-active substances (27). Based on the surface-active properties of ginsenosides, the foam floatation technique can be applied to the concentration and purification of the ginsenosides.

The purpose of the work is to develop a new degradation method for obtaining ginsenosides Rg₃, Rh₂ and 20(*S*)-protopanaxadiol. Both foam floatation and degradation conditions were investigated and optimized. Neutral solution, acidic solution, alkaline solution and *N,N*-dimethylformamide (DMF) were used as degradation media in the degradation of ginsenosides obtained from leaf of *Panax quinquefolium* L., respectively.

MATERIALS AND METHODS

Materials and Reagents. Ginsenosides Rg₁, Re, Rc, Rb₂, Rb₃, Rd, Rg₃, Rh₂ and aglycon 20(*S*)-protopanaxadiol were purchased from Chinese Medical and Biological Products Institute (Beijing, China). Chromatographic grade acetonitrile was obtained from Fisher Scientific Company (Pittsburgh, PA), and pure water was from a Milli-Q water

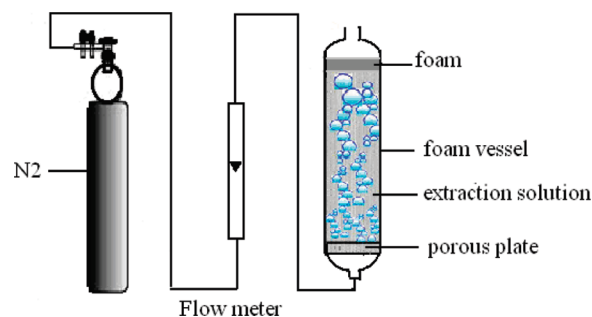


Figure 2. The setup of foam floatation.

purification unit (Millipore, Mississauga, Canada). Other reagents used in this study were of analytical grade.

Sample Preparation. Leaf of *Panax quinquefolium* L. was obtained from Jilin Province in China. The leaf was rinsed with water, dried at 30 °C, powdered in a mill, and passed through a 40-mesh sieve. Sample powder was weighed (1.0 g) and transferred into a 150 mL conical flask. 50 mL of ethanol–water solvent (70%, v/v) was added into it. The sample was subjected to ultrasonic extraction for 2 h. After the ultrasonic extraction was completed, the extract was filtered. The flask and residue were rinsed three times with ethanol. The collected extract and rinsed solvent were combined and evaporated to dryness under vacuum at 60 °C. Then the dried residue was dissolved in 1000 mL of water, and the resulting aqueous solution was referred to as extraction solution.

Foam Floatation of Ginsenosides. A 200 mL portion of the extraction solution was transferred to a floatation vessel (Figure 2), and then 2.0 g of NaCl was added into it. The first floatation for the extraction solution was carried out when the flow rate of carrier gas was maintained at 30 mL/min for 30 min. The foam phase obtained was diluted to 200 mL again with water. Under the same conditions mentioned above, the second floatation was carried out, and then the foam phase obtained was defoamed for about 10 min. The final volume of the foam phase was 20 mL. The solution resulting from the foam phase was referred to as floatation solution.

Microwave-Irradiation Degradation. Microwave-irradiation degradation was performed on a WRT-C microwave preparation system (Miche Technology Co. Ltd., Beijing, China) with a temperature control system. In the degradation in neutral solution, the floatation solution was transferred into the degradation vessel directly. The control vessel was operated by a control terminal that could control both temperature and irradiation time. After the degradation vessels and control vessel were put into the microwave irradiation system, the system was turned on and the degradation was continuously performed for 5 min under 165 °C. After the completion of degradation, the degradation vessels were allowed to cool down to the room temperature. Finally, the degradation products were extracted three times with 10 mL of water-saturated *n*-butanol at a time. The extracts were combined and evaporated to dryness under vacuum at 80 °C, and the residue was redissolved in 5 mL of methanol, which was referred to as degradation solution and filtered prior to HPLC–MS analysis.

In the degradation in acidic and alkaline solution, after the floatation solution was transferred into the degradation vessel, HCl and NaOH were added into it respectively. When the degradation temperature and time were fixed at 165 °C and 5 min respectively, the effect of concentrations of HCl ranging from 0.25 to 2 mol/L and concentrations of NaOH ranging from 0.1 to 1 mol/L were investigated. After the completion of degradation, the subsequent steps were the same as those applied in the neutral solution.

In an additional study, an organic medium was investigated. The floatation solution was extracted three times with 10 mL of water-saturated *n*-butanol at a time. Then the extracts were combined and evaporated to dryness under vacuum at 80 °C, and the dried residue was dissolved in 20 mL of DMF. After the DMF solution was transferred into the degradation vessel, the degradation process was performed under 165 °C for 10 min. After the completion of the degradation, the subsequent steps were the same as those applied in the neutral solution.

Determination by HPLC. The ginsenosides were quantitatively determined by liquid chromatography using an Agilent 1100 HPLC

Table 1. Calibration Curve and Concentration Range for Determination of Seven Ginsenosides

ginsenoside	regression equation ^a	correlation coeff	concn range	detection limit
			($\mu\text{g/mL}$)	($\mu\text{g/mL}$)
Rc	$A = 4434.37c + 5.223$	0.99974	2.3–147	0.5
Rb ₂	$A = 4654.77c + 12.21$	0.99998	8.7–559	2.2
Rb ₃	$A = 4700.31c + 33.42$	0.99985	9.8–629	2.6
Rd	$A = 6103.67c - 20.81$	0.99974	8.2–524	0.8
Rg ₃	$A = 8960.69c - 7.99$	0.99984	7.9–505	0.4
Rh ₂	$A = 9025.62c + 6.89$	0.99993	3.5–225	0.2
aglycon	$A = 8563.43c + 30.82$	0.99947	8.4–539	1.8

^aWhere A and c are the peak area and concentration of the analytes, respectively.

system (Palo Alto, CA) equipped with a Zorbax Eclipse XDB-C18 (250 mm $\times$ 4.6 mm i.d., 5 μm) column (Agilent, USA). The mobile phase was acetonitrile/water at a flow rate of 1.0 mL/min. The UV detection was performed at 203 nm, and the column temperature was 25 °C. The injection volume of sample solution was 20 μL . The mobile phase was a binary solvent containing acetonitrile (A) and water (B), and the gradient condition was as follows: 0–15 min, 24% A, 76% B; 15–40 min, 24–39% A, 76–61% B; 40–65 min, 39–53% A, 61–47% B; 65–85 min, 61–100% A, 47–0% B. The peak area was measured and used to construct the calibration curve.

HPLC–APCI/MS Analysis. The HPLC conditions for the HPLC–MS analysis were as described above. The effluent from chromatographic column was introduced into an ABI Q-Trap Mass Spectrometer (Applied Biosystems Sciex, Foster City, CA) equipped with atmospheric pressure chemical ionization source. Nitrogen (99.999%) was used as curtain gas, nebulizer gas and auxiliary gas. The curtain gas, nebulizer gas and auxiliary gas were 30, 35, and 40 psi respectively. The ion polarity was set to negative mode, and the ion source temperature was controlled at 350 °C. Needle current, ion spray voltage, entrance potential, declustering potential and collision energy were $-6\mu\text{A}$, 3000 V, -10 V , -50 V and -10 eV , respectively.

Degradation of Ginsenosides Standards. Ginsenoside standards Rc, Rb₂, Rb₃ and Rd (1.0 mg each) were placed in the degradation vessels and dissolved with 20 mL of sodium hydroxide solution (0.5 mol/L). Subsequently, the degradation was performed at 165 °C for 5 min. The degradation solution obtained was extracted three times with 10 mL of water-saturated *n*-butanol at a time. The extracts were combined and evaporated to dryness under vacuum at 80 °C. The residue was redissolved in 5 mL of methanol, and filtered prior to detection by HPLC–MS. For comparison, the ginsenoside standards were directly detected by APCI/MS.

Standard Curve for Quantitative Analysis. As shown in Table 1, standard curves were constructed for each of the ginsenoside standards (Rc, Rb₂, Rb₃, Rd, Rg₃, Rh₂ and aglycon) and used to determine the analytes in the degradation solution. Some ginsenosides in the degradation solution were identified by comparison of the retention times with those obtained from the mixed ginsenoside standards. The other ginsenosides were identified by matching of their mass spectral fragments with MS data reported in previously studies (28, 29).

Statistical Analysis of Experimental Data. In the study, each value reported is the mean of three replicate measurements.

The degradation yield Y of rare ginsenosides in microwave degradation is defined as follows:

$$Y = m_g/m_s \quad (1)$$

where m_g is the mass of rare ginsenosides obtained and m_s is the mass of sample powder.

Equation 2 was applied to calculating the transformation rate R of major ginsenosides to rare ginsenosides in microwave degradation:

$$R = M_r/M_m \quad (2)$$

When eq 2 was applied to calculating the transformation rate R of major ginsenosides in practical samples, M_r was the mole number of rare ginsenosides, including Rg₃, Rh₂ and aglycon, in the degradation solution

and M_m was the total mole number of major ginsenosides, including Rc, Rb₂, Rb₃ and Rd, in the floatation solution. When eq 2 was applied to calculating the transformation rate R of ginsenoside standards, M_r was the total mole number of the rare ginsenosides, including Rg₃, Rh₂ and aglycon, in the degradation solution and M_m was the mole number of the ginsenoside standard.

RESULTS AND DISCUSSION

The Foam Floatation of Ginsenosides. Some parameters affecting the performances of the foam floatation, such as the flow rate of carrier gas (N₂), floatation time, amount of sample, concentration of NaCl and pH value of extraction solution, were investigated. The amount of sample in 200 mL of extraction solution has a slight effect on foam floatation. The effect of the amount of sample ranging from 0.2 to 2.0 g on the recoveries was evaluated. The recoveries of protopanaxadiol type ginsenosides decrease with increasing amount of sample. Only in the case of Re and Rg₁, no effect was obvious. Therefore, 0.2 g sample in 200 mL of extraction solution was chosen. Sodium chloride, which was used as salting-out agent, was added into the extraction solution to improve the ionic strength. The recoveries were evaluated by varying the concentration of NaCl from 0 to 50 mg/mL. The recoveries of protopanaxatriol type ginsenosides decrease with increasing concentration of NaCl, and those of protopanaxadiol type are highest and constant at the concentrations ranging from 5 to 10 mg/mL. In addition, it can be found that when the concentration of NaCl is 10 mg/mL, the recoveries of protopanaxadiol type ginsenosides are highest and meanwhile there is almost no protopanaxatriol type ginsenoside in the solution. As a result, NaCl has a significant effect on the separation of protopanaxadiol type ginsenosides from protopanaxatriol type ginsenosides, and the concentration of NaCl was selected as 10 mg/mL in this work. The existing form of ginsenosides is related to the pH value of solution. In the pH range from 2 to 13, pH value has a significant effect on the recoveries for ginsenosides, especially for protopanaxadiol type ginsenosides. It is shown that the recoveries of all the ginsenosides are highest when the pH value is 9. However, because the pH of water used in this study was approximately 7–8 before floatation, no other reagents were added in order to simplify the experimental procedure. The flow rates of N₂ and the floatation times were investigated. When the flow rate of carrier gas increases, the amount of liquid entrained by the foam increases. As a result, the ginsenosides adsorbed in the foam are diluted. However, when the flow rate of carrier gas was too low, it took a long time to complete foam floatation. The compromising conditions selected are that the flow rate of carrier gas and floatation time are 30 mL/min and 30 min respectively. The foam floatation provides good recoveries (76.6–85.5%) for protopanaxadiol type ginsenosides, and the enrichment factors for Rc, Rb₂, Rb₃ and Rd are 7.66, 7.89, 8.55 and 8.50, respectively. However, for protopanaxatriol type ginsenosides, including Rg₁ and Re, the foam floatation does not provide good recoveries (11.1–15.3%) and enrichment factors (1.11–1.53). The foam floatation is more favorable to obtain Rg₃, Rh₂ and aglycon. Typical chromatograms of extraction and floatation solution are shown in Figure 3.

The Degradation of Ginsenosides. The main source of both ginsenosides Rg₃ and Rh₂ has been reported to be exclusively Korean red ginseng root which is steam-processed (30, 31). In this study, microwave irradiation degradation was applied in order to obtain rare ginsenosides. Both time and temperature of degradation have a significant effect on yields of rare ginsenosides, and different degradation media, including neutral solution, acidic solution, alkaline solution and DMF, were also investigated. The highest

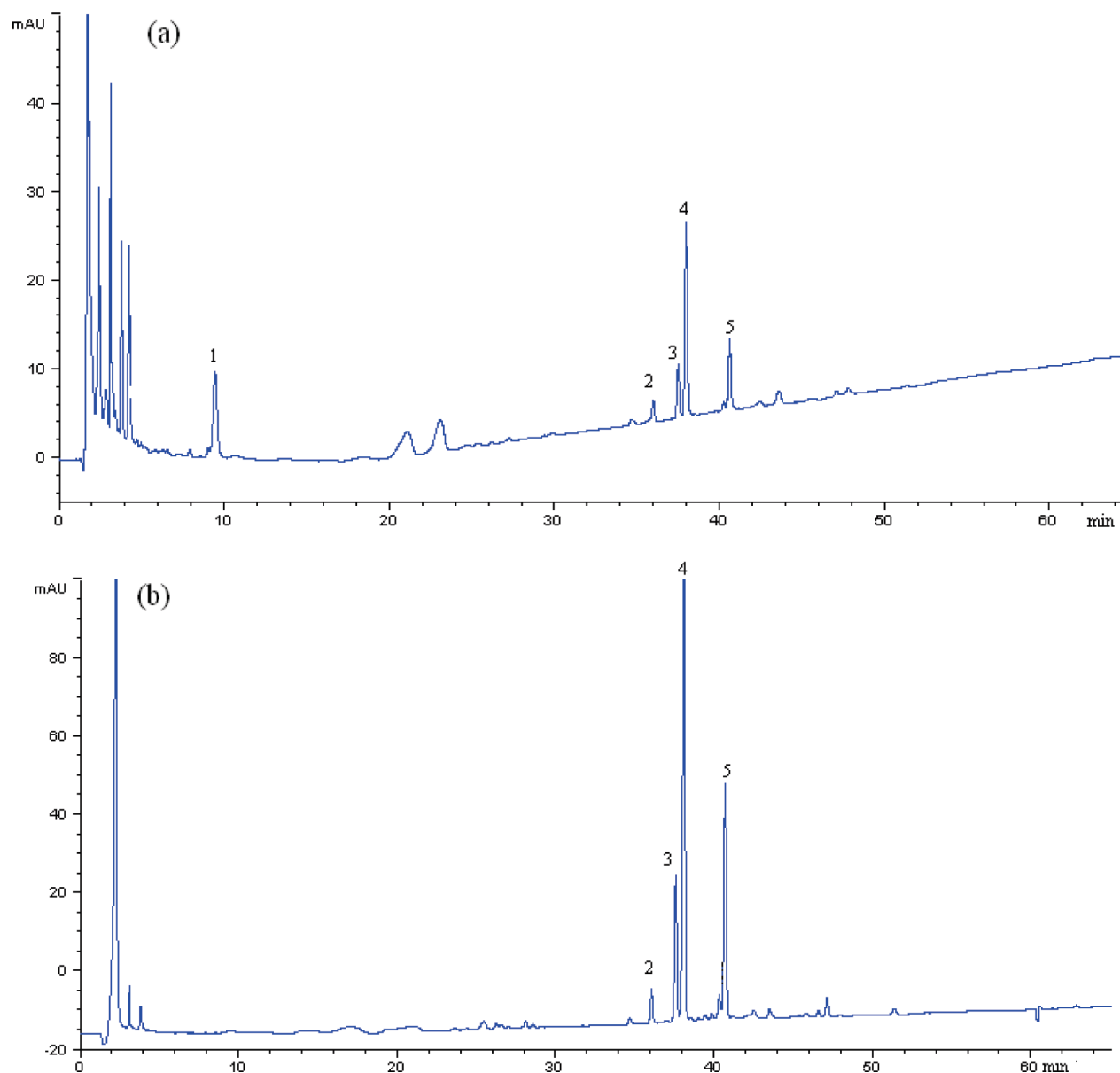


Figure 3. The chromatograms of extraction solution (a) and floatation solution (b): (1) Rg₁+Re; (2) Rc; (3) Rb₂; (4) Rb₃; (5) Rd.

Table 2. The Transformation Rates of Total Ginsenoside to Rare Ginsenosides

ginsenoside	transformation rate (%)			DMF
	neutral solution	acidic solution	alkaline solution	
Rg ₃	47.98	4.83	9.14	3.54
Rh ₂	6.43		6.94	
aglycon	14.98	26.92	78.11	20.20

yield of aglycon obtained under optimal conditions in alkaline medium was 4.83 mg/g, and the transformation rate of degradation of major ginsenosides to aglycon was 78.11% (Table 2).

In the degradation in neutral solution, the effect of the degradation temperatures ranging from 120 to 190 °C was investigated. The experimental results showed that the temperature had a significant effect on the yield of aglycon, which ranges from 0 to 0.43 mg/g. In the degradation solution, ginsenoside Rg₃, Rh₂, and aglycon were all detectable. As shown in Figure 4a, the yields of the three rare ginsenosides are highest at 165 °C. Too high or too low temperature is not favorable for the degradation yield, so 165 °C was chosen as the degradation temperature.

When the degradation temperature was kept at 165 °C, the effect of degradation times was investigated. From the Figure 4b, it can be seen that the yield of Rg₃ reaches the highest when the degradation time is 15 min. However, the yields of Rh₂ and aglycon increase with the increase of the degradation time ranging from 0 to 5 min, and no significant change was observed when the degradation time was longer than 5 min. Because the activity of aglycon is stronger than that of Rg₃ and Rh₂ (9, 13), the degradation time of 5 min was chosen to save time.

In the degradation in acidic solution, the degradation yield of aglycon decreases obviously with the increase of concentration of HCl ranging from 1.5 mol/L to 2.0 mol/L, and no significant change (0.40 mg/g) was observed when the concentration of HCl was lower than 1.5 mol/L (Figure 4c). In addition, ginsenoside Rh₂ was not obtained. Therefore, the concentration of HCl chosen was 1.5 mol/L in the present work.

In the degradation in alkaline solution, the effect of the concentration of NaOH is very significant in the generation of aglycon, with the yields changing from 0.09 to 4.83 mg/g (Figure 4d). The yield of Rg₃ first decreases with the increase of the concentration of NaOH ranging from 0.1 to 0.25 mol/L, and

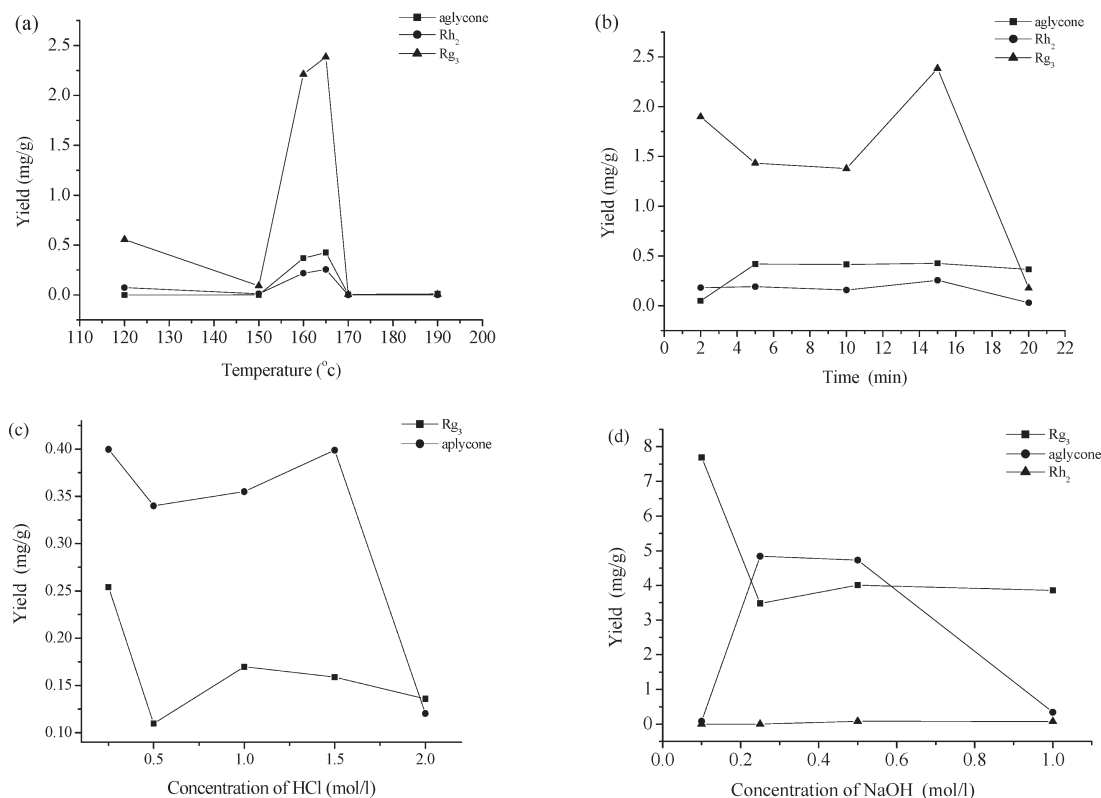


Figure 4. Influence of degradation temperature (a), degradation time (b), concentration of HCl (c) and concentration of NaOH (d) on yields of rare ginsenosides in H₂O.

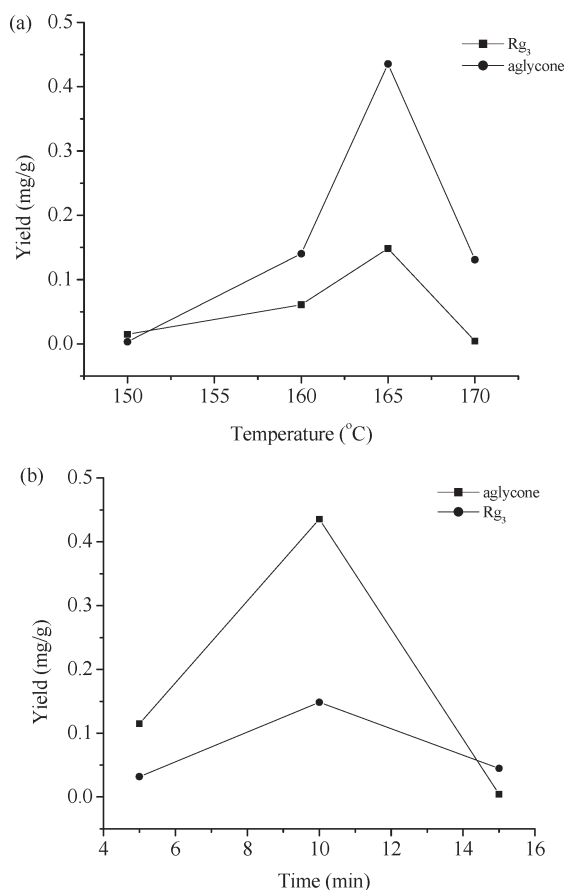


Figure 5. Influence of degradation temperature (a) and degradation time (b) on yields of rare ginsenosides in DMF.

then does not significantly change. The concentration of NaOH has a slight effect on the yield of Rh₂. As a result, 0.5 mol/L was selected as the concentration of NaOH.

Temperature has a significant effect on the yields of rare ginsenosides instead of pressure (30). Increasing temperature has been made at the expense of increasing pressure in water, so it is essential to introduce a suitable medium with high boiling point to accept microwave energy in which the ginsenosides can be degradable. Unlike studies reported (32, 33), we discovered that the ginsenosides were also degradable in *N,N*-dimethylformamide, which is a proton inert solvent with lower toxicity than pyridinium and lower pressure than water.

In the degradation in DMF, different degradation temperatures ranging from 150 to 170 °C were examined (Figure 5a). The results show that the temperature has a significant effect on the yield of aglycon which ranges from 0.003 to 0.44 mg/g. In the degradation solution, Rh₂ was not obtained and the yield of Rg₃ was quite low. So, 165 °C, which is the same as the temperature in degradation in neutral solution, was chosen.

The effect of degradation times ranging from 5 to 20 min was investigated when the degradation temperature was maintained at 165 °C. As shown in Figure 5b, degradation time has a significant effect on the yield of Rg₃ and aglycon. The yield of aglycon reaches the highest (0.44 mg/g) when the degradation time is 10 min. As a result, 10 min of degradation time was chosen.

HPLC–APCI/MS Analysis of Degradation Products. In order to identify ginsenosides in the degradation products, the degradation solutions were analyzed by HPLC–APCI/MS. APCI that could provide explicit information for identifying and distinguishing protopanaxadiol and protopanaxatriol type ginsenosides (26) has been considered to be mainly applicable to the assay of weakly polar compounds. Figure 6 shows the negative-ion HPLC–APCI/MS traces of ginsenosides in the degradation solution. It can be seen that the target compounds in the

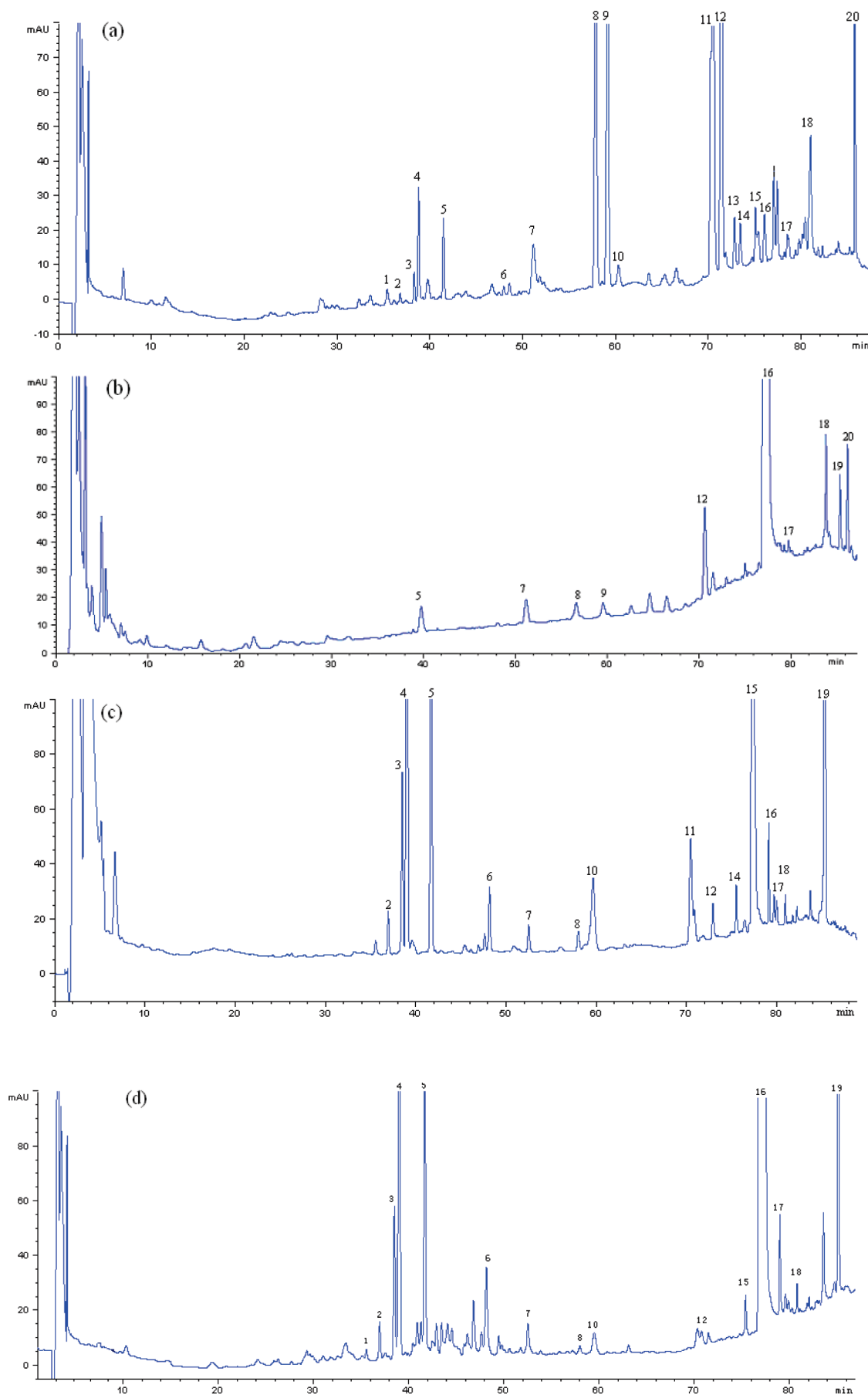


Figure 6. HPLC chromatogram for degradation solutions obtained in neutral solution (a), acidic solution (b), alkaline solution (c) and DMF (d): (1) Rb₁; (2) Rc; (3) Rb₂; (4) Rb₃; (5) Rd; (6) Rd₂; (7) F₂; (8) 20(S)-Rg₃; (9) 20(R)-Rg₃; (10) not reported; (11) not reported; (12) Rg₅; (13) 20(S)-Rh₂; (14) 20(R)-Rh₂; (15) Rk₁/Rs₄; (16) Rk₁/Rs₄; (17) not reported; (18) not reported; (19) 20(S)-protopanaxadiol; (20) 20(R)-protopanaxadiol.

Table 3. HPLC–APCI/MS Data of Ginsenosides in Degradation Solutions

peak	identification	retention time (min)	main fragmentation ion <i>m/z</i>		others			
			[M − H] [−]	[M + HCOO] [−]				
Neutral ^a								
1	Rb ₁	34.97	1077.9	1123.8	765.5	699.5	475.4	373.5
2	Rc	36.56	1077.9	1123.8	765.5	699.5	475.4	373.5
3	Rb ₂	38.18	1077.9	1123.8	765.5	699.5	475.4	373.5
4	Rb ₃	38.67	1078.0	1124.0	765.5	699.7	667.6	621.7
5	Rd	41.36	945.8	991.9	765.5	699.7	667.9	621.7
6	Rd ₂	48.28	915.8	961.6	683.7	667.8	649.7	621.7
7	F ₂	52.60	783.9	829.5	649.8	667.6	493.5	
8	20(<i>S</i>)-Rg ₃	57.60	783.9	829.5				
9	20(<i>R</i>)-Rg ₃	59.59	783.9	829.5				
10	not reported	60.47						
12	Rg ₅	71.14	765.4		621.7	459.7		
13	20(<i>S</i>)-Rh ₂	72.65	621.5	667.5	493.6	475.8		
14	20(<i>R</i>)-Rh ₂	73.19	621.5	667.5	493.6	475.8		
15	Rk ₁ /Rs ₄	76.11	621.5	667.5	459.7			
16	Rk ₁ /Rs ₄	77.84	621.5	667.5	459.7			
17	not reported	79.65	765.4					
18	not reported	80.53	649.4	668.7	545.9			
20	20(<i>R</i>)-protopanaxadiol	86.07	459.7	505.4				
Acidic ^a								
5	Rd	41.36	945.8	991.9	765.5	699.7	667.9	621.7
7	F ₂	52.60	783.9	829.5	649.8	667.6	493.5	
8	20(<i>S</i>)-Rg ₃	57.60	783.9	829.5				
9	20(<i>R</i>)-Rg ₃	59.59	783.9	829.5				
12	Rg ₅	71.14	765.4		621.7	459.7		
16	Rk ₁ /Rs ₄	77.84	621.5	667.5	459.7			
17	not reported	79.65	765.4					
18	not reported	80.53	649.4	668.7	545.9			
19	20(<i>S</i>)-protopanaxadiol	85.30	459.7	505.4				
20	20(<i>R</i>)-protopanaxadiol	86.07	459.7	505.4				
Alkaline ^a								
1	Rb ₁	34.97	1107.9	1123.8	765.5	699.5	475.4	373.5
2	Rc	36.56	1077.9	1123.8	765.5	699.5	475.4	373.5
3	Rb ₂	38.18	1077.9	1123.8	765.5	699.5	475.4	373.5
4	Rb ₃	38.67	1078.0	1124.0	765.5	699.7	667.6	621.7
5	Rd	41.36	945.8	991.9	765.5	699.7	667.9	621.7
6	Rd ₂	48.28	915.8	961.6	683.7	667.8	649.7	621.7
7	F ₂	52.60	783.9	829.5	649.8	667.6	493.5	
8	20(<i>S</i>)-Rg ₃	57.60	783.9	829.5				
10	not reported	60.47						
12	Rg ₅	71.14	765.4		621.7	459.7		
13	20(<i>S</i>)-Rh ₂	72.65	621.5	667.5	493.6	475.8		
15	Rk ₁ /Rs ₄	76.11	621.5	667.5	459.7			
16	Rk ₁ /Rs ₄	77.84	621.5	667.5	459.7			
17	not reported	79.65	765.4					
18	not reported	80.53	649.4	668.7	545.9			
19	20(<i>S</i>)-protopanaxadiol	85.30	459.7	505.4				
DMF ^a								
1	Rb ₁	34.97	1077.9	1123.8	765.5	699.5	475.4	373.5
2	Rc	36.56	1077.9	1123.8	765.5	699.5	475.4	373.5
3	Rb ₂	38.18	1077.9	1123.8	765.5	699.5	475.4	373.5
4	Rb ₃	38.67	1078.0	1124.0	765.5	699.7	667.6	621.7
5	Rd	41.36	945.8	991.9	765.5	699.7	667.9	621.7
6	Rd ₂	48.28	915.8	961.6	683.7	667.8	649.7	621.7
7	F ₂	52.60	783.9	829.5	649.8	667.6	493.5	
8	20(<i>S</i>)-Rg ₃	57.60	783.9	829.5				
10	not reported	60.47						
12	Rg ₅	71.14	765.4		621.7	459.7		
15	Rk ₁ /Rs ₄	76.11	621.5	667.5	459.7			
16	Rk ₁ /Rs ₄	77.84	621.5	667.5	459.7			
17	not reported	79.65	765.4					
18	not reported	80.53	649.4	668.7	545.9			
19	20(<i>S</i>)-protopanaxadiol	85.30	459.7	505.4				

^a Degradation solution.

degradation solution were well separated under the HPLC conditions adopted. Based on retention times and the m/z values of both intact molecular ions and the product ions, ginsenosides labeled on the reconstructed ion chromatograms were identified. The retention times and the set of ions for individual ginsenosides in the four degradation solutions are listed in **Table 3**. From **Figure 6**, it can be seen that ginsenoside Rg_3 and aglycon are detectable in all the degradation solutions and ginsenoside Rh_2 , another rare ginsenoside, is detectable only in alkaline and neutral degradation solutions. In the neutral degradation solution, the isomeric compounds of Rg_3 and Rh_2 , including $20(R)$ and $20(S)$ configuration, are generated simultaneously at almost the same concentration. Furthermore, the configuration of aglycon obtained in neutral degradation solution was only $20(R)$ (**Figure 6a**). In the acidic degradation, compared with the result of earlier study (20), the ginsenosides were completely degraded and only a small amount of aglycon, of which the concentration of $20(R)$ is more than that of $20(S)$, was obtained (**Figure 6b**). On the contrary, impurities in alkaline and DMF degradation solution are less than those obtained in the other two media and the

configuration of degradation products is only $20(S)$, which has stronger pharmaceutical activity than $20(R)$ (**Figures 6c** and **6d**).

Degradation Products of Ginsenoside Standards. The degradation yields of rare ginsenosides including Rc , Rb_2 , Rb_3 and Rd are shown in **Table 4**. Unlike the results reported (29), ginsenoside Rh_2 is the only degradation product of Rc with yield of 0.19 mg/mg. Moreover, ginsenoside Rg_3 is the only degradation product of Rd with the yield of 0.13 mg/mg. However, aglycon was generated from all four major ginsenosides but with the highest yield from Rd . The difference may be due to the difference in heating modes and degradation media.

Degradation Mechanism. For the protopanaxadiol type ginsenosides with β -OH moiety at C_3 , C_{12} and C_{20} , when the glycosidic bond at the C-20 position is hydrolyzed, the glycosidic bond at the C-3 position is also attacked (34, 35).

During the degradation of ginsenosides in the strongly acidic solution, no aglycon but some byproduct can be obtained, which is mainly because the side chain moiety of the ginsenoside molecular structure is easily cyclized in the strongly acidic solution.

When the four degradation media were applied in the degradation of ginsenosides, the generation reactions of rare ginsenosides were as shown in **Figure 7**, taking the neutral solution as an example. The increase of Rh_2 and aglycon usually goes with the decrease of Rg_3 , but the results obtained in this paper indicate that the generation tendency of three rare ginsenosides is the same, so it can be speculated that the three ginsenosides are generated simultaneously in the method used in this paper. The possible reason is that the energy offered from microwave irradiation is so intensive that the hydrolyzation of glycosidic bonds at the C_3 , C_{12} and C_{20} positions is accelerated and occurs simultaneously.

Conclusion. Microwave irradiation degradation coupled with foam floatation was developed to prepare the rare ginsenosides Rg_3 , Rh_2 and aglycon. Foam floatation prior to degradation of ginsenosides is favorable to the concentration and purification of the ginsenosides of interest. Owing to its simplicity, low operation cost, few impurities and particularly high yield and the saving in time, the proposed method can be applied successfully to the degradation of major ginsenosides in order to obtain rare

Table 4. Yields for Rg_3 , Rh_2 and Aglycon Obtained from Ginsenoside Standards

ginsenoside	target degradation product	yield (mg/mg)	transformation rate (%)
Rc	Rg_3	0.00	39.96
	Rh_2	0.19	
	aglycon	0.03	
Rb_2	Rg_3	0.00	4.69
	Rh_2	0.00	
	aglycon	0.02	
Rb_3	Rg_3	0.00	4.69
	Rh_2	0.00	
	aglycon	0.02	
Rd	Rg_3	0.20	96.11
	Rh_2	0.00	
	aglycon	0.35	

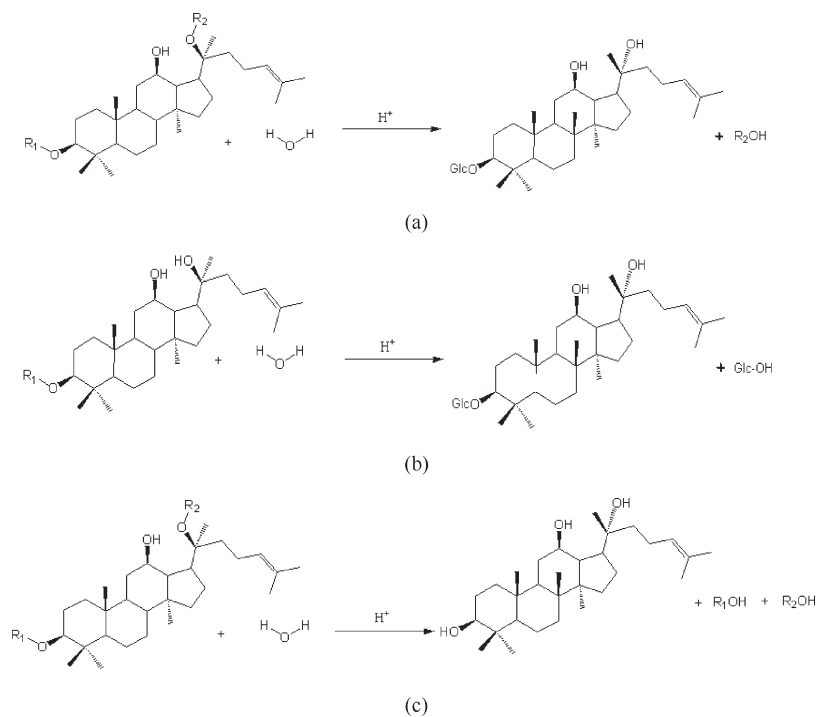


Figure 7. The generation of rare ginsenosides Rg_3 (a), Rh_2 (b) and aglycon (c) in neutral solution.

ginsenosides. Considering leaf material is accessible during the entire plant cycle, it is an important finding that the leaf of *Panax quinquefolium* L. processed by microwave irradiation degradation coupled with foam floatation is a new excellent source of rare ginsenosides. This method is therefore valuable for the generation of rare ginsenosides and may be a good alternative to the traditional techniques.

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