Degradation of Ginsenosides in American Ginseng (*Panax quinquefolium*) Extracts during Microwave and Conventional Heating

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The degradation of ginsenosides in American ginseng (*Panax quinquefolium*) extracts during microwave and water (oil) bath heating (conventional heating) was investigated. Both the 50% ethanol−water extracts and the aqueous extracts were boiled in a modified laboratory microwave oven and in a water (or oil) bath, respectively. The neutral ginsenosides (Rb_1, Rc, Rd, and Re) and malonyl ginsenosides (m-Rb_1, m-Rc, and m-Rd) were determined by reverse-phase high-performance liquid chromatography. The results showed that the degradation of ginsenosides in 50% ethanol−water extracts was a first-order reaction. The malonyl ginsenosides were much less stable than the corresponding neutral ginsenosides, with the rate constant value of the malonyl ginsenosides being 3−60 times that of the neutral ginsenosides. At the same temperature, the effect of microwave heating on the degradation of ginsenosides was the same as that of conventional heating.

Keywords: *Panax quinquefolium; ginsenosides; degradation; heating; microwave*

INTRODUCTION

Both Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolium*) are very important medicinal plants. Their roots are used as a traditional Chinese medicine and health food (Carlson, 1986; Proctor et al., 1988; Li, 1995). Worldwide ginseng production was valued at about U.S. $600 million in 1991 (But et al., 1995; Liu and Lui, 1995; Park and Kim, 1995). Ginseng products are increasingly popular and readily available in pharmacies and health food stores around the world.

Ginseng extract is one of the main ginseng products. Usually, it is made by extracting ginsenosides from ginseng powder with water or aqueous ethanol under water (oil) bath heating (conventional heating) (Sung et al., 1981, 1985).

Ginsenosides are the main active constituents of both Asian ginseng and American ginseng, which include neutral ginsenosides and malonyl ginsenosides. They have many important biological and pharmacological activities, including antitumor, chemopreventive, antiphlogistic, immunomodulating, and antidiabetic activities and activities on the cardiovascular system, the central nervous system, and the endocrinal system (Lacaille-Dubois and Wagner, 1996; Gillis, 1997). The most abundant ginsenosides present in Asian ginseng and American ginseng are neutral ginsenosides, Rb_1, Rb_2, Rc, Rd, Re, and Rg_1, and malonyl ginsenosides, m-Rb_1, m-Rb_2, m-Rc, and m-Rd, and substantial amounts of Rf are also found in Asian ginseng, but not in American ginseng (Chuang et al., 1995; Ko et al., 1995).

Ginsenosides, especially malonyl ginsenosides, are thermally unstable; they may degrade during thermal extraction (Yamaguchi et al., 1988; Samukawa et al., 1995a,b). Thermal degradation of neutral ginsenosides in aqueous solution during conventional heating has been extensively studied (Choi et al., 1982; Sung and Yang, 1986; Lee et al., 1994). However, very little work has been done on the degradation of malonyl ginsenosides during conventional heating or on the degradation of ginsenosides in aqueous ethanol solution during heating.

Ginseng extraction by conventional heating is a very slow process (Ryu et al., 1979; Sung et al., 1985). Recently, microwave heating has been employed for the extraction of natural products, in which faster extraction rates were observed under certain conditions (Chen and Spiro, 1994, 1995; Spiro and Chen, 1995). Therefore, it is likely that microwave heating may also enhance the extraction of ginsenosides. Nonthermal effects during microwave heating were claimed by many researchers (Majetich and Hicks, 1995a,b). In other words, at the same temperature, the degradation of many nutritional components during microwave heating is greater than during conventional heating, so the nonthermal effects must be considered when microwave energy is used for certain food processing.

The aims of this work were to compare the degradation rates of ginsenosides from American ginseng extracts under microwave and conventional heating and to investigate the degradation of neutral ginsenosides and malonyl ginsenosides in the 50% ethanolic extracts at boiling point and the degradation of malonyl ginsenosides in the aqueous extracts at boiling point. This information is essential for the production of high-quality ginseng extracts.

EXPERIMENTAL PROCEDURES

American Ginseng Roots. Fresh American ginseng (*P. quinquefolium*) roots were obtained from the Institute of Special Plants and Wild Animals, Chinese Academy of Agricultural Sciences, Jilin City, People's Republic of China. The

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fresh roots were, subsequently, shipped to Hong Kong and dried at 38 °C until the moisture content of the roots was ~10%. Dried American ginseng roots were cut into small pieces, then ground with a blender, and screened using a 200 mesh sieve.

American Ginseng Aqueous Extracts. The ground American ginseng sample (400 g) was transferred to a 2.5-L flask containing 2 L of distilled water, the pH value of which was 6.83. Samples were stirred occasionally at room temperature for 12 h to ensure optimal extraction. The mixture was then transferred to centrifuge tubes and centrifuged at 4500 rpm for 15 min. The extracts were finally filtered through Whatman No. 1 filter paper. The pH value of the extracts was 4.95.

American Ginseng Ethanol Extracts. The preparation method was the same as above except 50% ethanol−water was used as the solvent instead of distilled water. The pH value of the extracts was 5.54.

Microwave Heating. The microwave heating system used consisted of two parts—a laboratory microwave oven and a modified reflux system. The laboratory microwave oven (Lavis, 100 MultiQUANT) operated at 2450 MHz. The energy emission was microprocessor controlled from 10 to 1000 W at 10-W increments. Three outlets were provided on the left upper side of the oven to allow introduction of temperature sensors, and another one was on the right top of the oven to allow introduction of a simple reflux system. The dimensions of the microwave cavity were 345 mm × 340 mm × 225 mm. The microwave oven was operated by a control terminal that could control both microwave power level and emission time (1 s−100 h). Modification of the reflux system was made to facilitate sampling and reduce the possibility of ethanol loss due to flash evaporation. Three independent runs were performed at a microwave power level of 200 W. After the boiling point had been reached, initial samples of American ginseng extracts were taken. All of the samples were stored in a freezer (−20 °C). Prior to the ginsenoside analysis, the samples were centrifuged at 13000 rpm for 10 min.

Conventional Heating. Conventional heating experiments were performed in a water bath and oil bath with reflux systems for aqueous ethanol American ginseng extracts and aqueous American ginseng extracts, respectively.

High-Performance Liquid Chromatography (HPLC). HPLC was conducted on a Waters liquid chromatograph equipped with two 510 pumps and a UV spectrophotometric detector. The American ginseng extract solution was separated and analyzed (20-μL aliquots) by using a Merck Superspher RP-18 end-capped column (250 × 4.0 mm; 5 μm) at room temperature. The mobile phase consisted of solvent A (acetonitrile) and solvent B (phosphate buffer solution). Solvent B was prepared by dissolving 2.80 g of KH_2PO_4 in 2000 mL of water and adjusting the pH to 5.81 with a concentrated solution of K_2HPO_4 (35 g/100 mL). For the simultaneous separation of neutral ginsenosides and malonyl ginsenosides, the following gradient procedure was used: 0−25 min, 20−25% A, 80−75% B; 25−37 min, 25−32% A, 75−68% B; 37−50 min, 32−40% A, 68−60% B; 50−52 min, 40−100% A, 60−0%B; 62−65 min, 100−20%A, 0−80% B. The flow rate was kept constant at 1.0 mL/min. The absorbance was measured at a wavelength of 203 nm to facilitate the detection of ginsenosides. Chromatographic peaks were identified by comparing retention times against known standards or by comparing their retention times with published data (Chuang et al., 1994, 1995; William et al., 1996). The standard ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 were purchased from Extrasynthese (Genay, France), and ginsenoside Ro was obtained from the Institute of Special Plants and Wild Animals, Chinese Academy of Agricultural Sciences, Jilin City, People's Republic of China.

Rate Constant. Equation 1 was used to describe degradation kinetics of ginsenosides during conventional heating and microwave heating:

$$C = C_0 \exp(-kt) \quad (1)$$

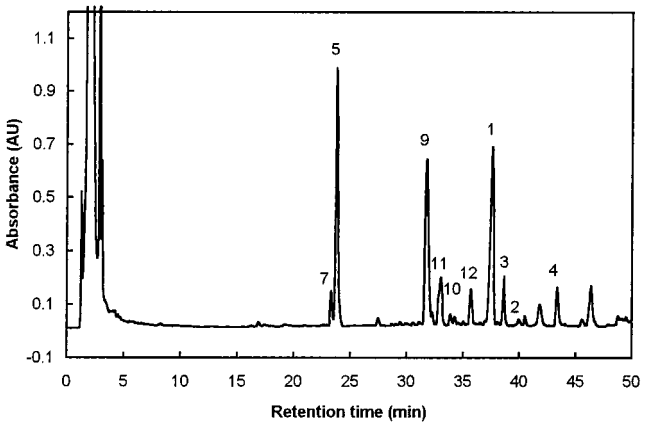

Figure 1. HPLC chromatogram of ginsenosides in aqueous ethanol extracts of *P. quinquefolium*: Rb_1 (1), Rb_2 (2), Rc (3), Rd (4), Re (5), Rg_1 (7), m-Rb_1(9), m-Rb_2 (10), m-Rc (11), m-Rd (12).

In eq 1, C is the concentration of solution at time t expressed by percentage (%), C_0 is the concentration of solution at time $t = 0$ expressed by percentage (%), k is a rate constant, and t is time.

Statistical Analysis of Experimental Data. Each reported value is the mean of three duplications. A modified LSD test was applied to determine significance between means, at a level of $p < 0.05$.

RESULTS AND DISCUSSION

For comparative study, temperature control is very important. The temperature in the reaction medium during both conventional and microwave heating must be controlled at the same level. However, during microwave heating, temperature control has proven to be very difficult when reactions are being carried out at a temperature below the boiling point (Galema, 1997). In this experiment, the reaction medium was heated to boiling and then kept at that temperature until the end of the experiment. The boiling point of the water extract was ~100 °C, and the boiling point of the 50% ethanol−water extract was ~85 °C. For the aqueous extract (100 mL), it took 7.5 min to reach the boiling point under conventional heating, whereas it took only 3 min to reach the boiling point under microwave heating (200 W). For the ethanol−water extract (100 mL), it took 6 min to reach the boiling point under conventional heating, whereas it took only 2 min to reach the boiling point under microwave heating. The initial sample for malonyl ginsenoside degradation study was taken immediately after the temperature had reached the boiling point. Hence, differences in degradation due to temperature profiles could be reduced. Sampling may disturb the temperature profile; however, the influence should be very little, because the sampling time was very short compared with the sampling interval (30 s versus 0.5 h). Therefore, the influence may be neglected.

As shown in Figure 1, the chromatographic separation of both the malonyl ginsenosides and the neutral ginsenosides in a single run was achieved by linear gradient elution. The peaks of six neutral ginsenosides and four malonyl ginsenosides were well resolved. In agreement with published results (Chuang and Sheu, 1994; Chuang et al., 1995; William et al., 1996), the protopanaxtriol derivatives Rg_1 and Re eluted ahead of the malonyl ginsenosides, m-Rb_1, m-Rc, m-Rb_2, and m-Rd, and the protopanaxdiol derivatives Rb_1, Rb_2, Rc, and Rd eluted last. Figure 2 shows the HPLC chro-

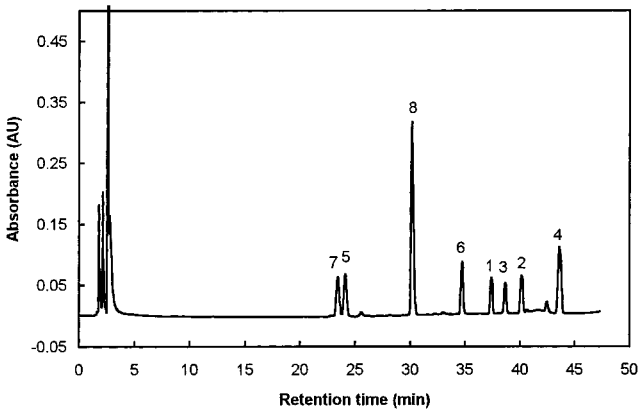

Figure 2. HPLC chromatogram of a mixture of standard ginsenosides: Rb_1 (1), Rb_2 (2), Rc (3), Rd (4), Re (5), Rf (6), Rg_1 (7), Ro (8).

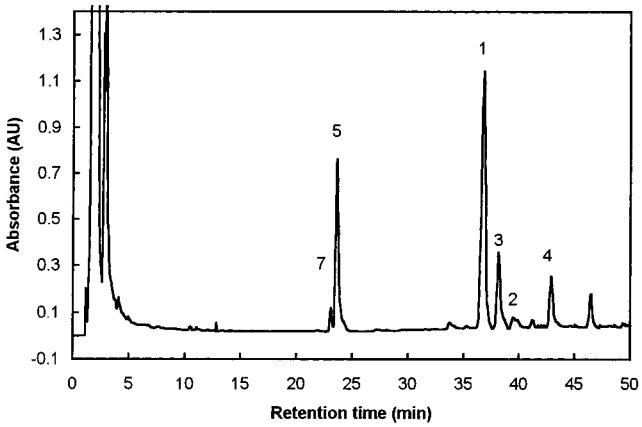

Figure 3. HPLC chromatogram of ginsenosides in aqueous ethanol extracts of *P. quinquefolium* after 9 h of heating: Rb_1 (1), Rb_2 (2), Rc (3), Rd (4), Re (5), Rg_1 (7).

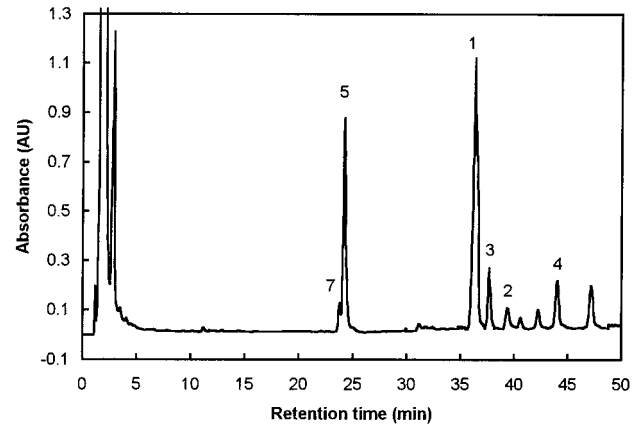

Figure 4. HPLC chromatogram of ginsenosides in aqueous extracts of *P. quinquefolium* after 3 h of heating: Rb_1 (1), Rb_2 (2), Rc (3), Rd (4), Re (5), Rg_1 (7).

matogram of a mixture of standard ginsenosides.

Malonyl ginsenosides are rather unstable, being readily demalonylated on heating. During heating, the concentration of malonyl ginsenosides decreased while the corresponding neutral ginsenosides increased. After 9 h of heating, no malonyl ginsenosides in the aqueous ethanol extract could be detected (Figure 3). In aqueous extracts, the time for all malonyl ginsenosides to decrease to undetectable levels was only 3 h (Figure 4). The initial samples for neutral ginsenoside degradation study in aqueous ethanol and aqueous extracts were taken after 9 and 3 h of heating, respectively, so the degradation kinetics of neutral ginsenosides did not interfere with the malonyl ginsenosides.

Table 1. Degradation of Ginsenosides from American Ginseng Extracts in 50% Ethanol Solution under Conventional Heating (CH) and Microwave Heating (MH) at the Boiling Point

ginsenoside	heating method	reaction order	rate constant[a] k ($\times 10^{-3}$, h^{-1})	$p = 0.05$[b]	correl coeff[a] (R^2)
Rb_1	CH	1	3.14	a	0.9947
	MH	1	3.05	a	0.9824
Rc	CH	1	3.42	b	0.9829
	MH	1	3.57	b	0.9357
Rd	CH	1	2.63	c	0.9742
	MH	1	2.48	c	0.9775
Re	CH	1	3.98	d	0.9224
	MH	1	4.16	d	0.9048
m-Rb_1	CH	1	181.36	e	0.9993
	MH	1	184.52	e	0.9862
m-Rc	CH	1	206.83	f	0.9986
	MH	1	197.22	f	0.9825
m-Rd	CH	1	165.96	g	0.9948
	MH	1	167.21	g	0.9783

[a] Mean value of three determinations, CV $\leq$ 10%. [b] Using modified LSD (Bonferroni) test. Values having the same letter in the column were not significantly different from each other at the level indicated by the p value. Values having different letters were significantly different at the level indicated by the p value.

Table 1 shows the degradation of ginsenosides in the 50% ethanol−water solution under conventional and microwave heating at the boiling point. The degradation of neutral and malonyl ginsenosides followed a first-order reaction, which was the same as the neutral ginsenoside degradation in water extracts (Choi et al., 1982; Lee et al., 1994). Most of the reactions involved in the processing of foods belong to first-order reactions (Holdsworth, 1985). The stability of ginsenosides varied depending on the sorts of ginsenosides. Neutral ginsenosides were more stable than malonyl ginsenosides. The rate constants of neutral ginsenoside were much lower than those of the malonyl ginsenosides. For example, the rate constant of the malonyl ginsenoside Rb_1 was 65.87 times that of the corresponding neutral ginsenoside Rb_1. Among the neutral ginsenosides, protopanaxdiol derivatives were more stable than protopanaxtriol derivatives. Rate constants of protopanaxdiol derivatives Rb_1, Rc, and Rd were significantly smaller than those of the protopanaxatriol derivative Re ($p < 0.05$). Among the protopanaxadiol derivatives, ginsenoside Rd was more stable than the others ($p < 0.05$). These results are in good agreement with those reported by others in aqueous solution (Choi et al., 1982; Sung and Yang, 1986). The rate constants of neutral ginsenosides in 50% ethanol−water solution at the boiling point were very small. This suggested that ethanol may be more suitable as a solvent for the extraction of ginsenosides. We can thus deduce that constants of ginsenosides in the aqueous ethanol extract at room temperature are also smaller. Usually, the Q_{10} value in food processing is ~2 (Toledo, 1991). When the reaction temperature drops from the boiling point (85 °C) to room temperature (25 °C), the rate constant may decrease to 1/64 of the original. This may provide a good explanation of the general processing method for traditional ginseng products such as ginseng alcohol. Ginsenosides in ginseng roots diffuse into alcohol, which can prevent ginseng from rotting and prevent ginsenosides from degrading rapidly. Results of the rate constants of

Table 2. Degradation of Ginsenosides from American Ginseng Extracts in Aqueous Solution under Conventional Heating (CH) and Microwave Heating (MH) at the Boiling Point

ginsenoside	heating method	reaction order	rate constant[a] k (h^{-1})	$p = 0.05$[b]	correl coeff[a] (R^2)
Rb_1	CH	1	0.2146	a	0.9785
	MH	1	0.2017	a	0.9923
Rc	CH	1	0.3083	b	0.9835
	MH	1	0.3124	b	0.9723
Rd	CH	1	0.1648	c	0.9486
	MH	1	0.1692	c	0.9829
Re	CH	1	0.3478	d	0.9258
	MH	1	0.3641	d	0.9126
m-Rb_1	CH	1	0.8376	e	0.9973
	MH	1	0.8532	e	0.9964
m-Rc	CH	1	0.9774	f	0.9828
	MH	1	1.0648	f	0.9935
m-Rd	CH	1	0.7022	g	0.9885
	MH	1	0.7439	g	0.9937

[a] Mean value of three determinations, CV ≤ 10%. [b] Using modified LSD (Bonferroni) test. Values having the same letter in the column were not significantly different from each other at the level indicated by the p value. Values having different letters were significantly different at the level indicated by the p value.

ginsenoside degradation during microwave and conventional heating indicated that there was no difference between the two heating methods ($p < 0.05$). A similar conclusion was also drawn in thiamin degradation (Welt and Tong, 1993).

Table 2 shows the degradation of ginsenosides in aqueous extracts under conventional and microwave heating. The reaction under conventional heating was first order, which is the same as that reported by others (Choi et al., 1982; Lee et al., 1994). The degradation of ginsenosides in aqueous extracts during microwave heating was a first-order reaction also. The rate constants of neutral ginsenosides Rb_1, Rc, and Rd were similar to those reported by others (Choi et al., 1982), whereas the rate constants of the malonyl ginsenosides were much larger than those of the neutral ginsenosides. Results of the rate constants of ginsenosides also indicated there was no significant difference between the conventional and microwave heating methods ($p < 0.05$).

CONCLUSION

The degradation of ginsenosides in ethanol solution follows a first-order reaction. Malonyl ginsenosides are more unstable than the corresponding neutral ginsenosides; the rate constants of malonyl ginsenosides are 3−60 times higher than those of the neutral ginsenosides. At the same temperature, the values of the rate constants of ginsenoside degradation in both aqueous and 50% ethanol−water extracts between the microwave and conventional heating methods were similar. Therefore, the attractive heating characteristics of microwave energy may be applied to the extraction of ginsenosides and thermal sterilization of the ginseng products.

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