



Research article

Influence of organic acids and heat treatment on ginsenoside conversion

Gwi Yeong Jang¹, Min Young Kim¹, Yoon Jeong Lee¹, Meishan Li¹, Yu Su Shin², Junsoo Lee¹, Heon Sang Jeong^{1,*}¹ Department of Food Science and Biotechnology, Chungbuk National University, Cheongju, Republic of Korea² Department of Medicinal Crop Research, National Institute of Horticultural and Herbal Science, Rural Development Administration, Eumseong, Republic of Korea

ARTICLE INFO

Article history:

Received 12 May 2017

Received in Revised form

3 July 2017

Accepted 20 July 2017

Available online 26 July 2017

Keywords:

ginseng
ginsenoside conversion
heat treatment
organic acids
thermal stability

ABSTRACT

Background: Heat treatments are applied to ginseng products in order to improve physiological activities through the conversion of ginsenosides, which are key bioactive components. During heat treatment, organic acids can affect ginsenoside conversion. Therefore, the influence of organic acids during heat treatment should be considered.

Methods: Raw ginseng, crude saponin, and ginsenoside Rb₁ standard with different organic acids were treated at 130°C, and the chemical components, including ginsenosides and organic acids, were analyzed. **Results:** The organic acid content in raw ginseng was 5.55%. Organic acids were not detected in crude saponin that was not subjected to heat treatment, whereas organic acids were found in crude saponin subjected to heat treatment. Major ginsenosides (Rb₁, Re, and Rg₁) in ginseng and crude saponin were converted to minor ginsenosides at 130°C; the ginsenoside Rb₁ standard was very stable in the absence of organic acids and was converted into minor ginsenosides in the presence of organic acids at high temperatures.

Conclusion: The major factor affecting ginsenoside conversion was organic acids in ginseng. Therefore, the organic acid content as well as ginsenoside content and processing conditions should be considered important factors affecting the quality of ginseng products.

© 2017 The Korean Society of Ginseng, Published by Elsevier Korea LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Panax ginseng Meyer has been used as a medicinal agent for thousands of years, and is a well-known herbal medicine and functional food [1]. Ginseng contains various functional components [2], and ginsenosides are the key bioactive components; they are composed of a dammarane skeleton with sugar moieties, such as glucose, rhamnose, xylose, and arabinose, at the C-3, C-6, and C-20 positions [3,4]. Major saponins identified in fresh ginseng include ginsenoside Rb₁, Rb₂, Rd, Rg₁, Re, and Rf [5]; however, minor ginsenosides such as Rg₃, Rh₂, Rh₄, Rs₃, Rs₄, and Rg₅ are characteristic components in processed ginsengs such as the red, black, and fermented ginsengs [6,7].

In general, ginsenosides have different physiological activities and bioavailabilities, and the biological activity of ginseng and ginseng products varies according to the ginsenoside composition.

For this reason, considerable efforts have been put forth toward improving the biological activities of ginseng using various conversion methods. There are three different ginsenoside conversion strategies (i.e., physical, chemical, and biological treatments), and physicochemical methods are commonly used in the industry for economic reasons. The chemical structures of ginsenosides may change upon hydrolysis of the sugar moieties and dehydration at C-20 [8], and the transformation pathways of ginsenosides by steaming process were proposed by Liu et al. [9] (Fig. 1).

Research studies on the effects of repeated steaming on ginsenoside composition and physicochemical properties of ginseng [10], changes in ginsenosides from black ginseng prepared due to steam–dry cycles [11], pH and temperature on ginsenoside composition in red ginseng water extracts [12], high-pressure and steaming extraction on ginsenoside Rg₃ and Rh₂ contents [13], high pressures, temperatures, and extraction solvents on ginsenosides of

* Corresponding author. Department of Food Science and Biotechnology, Chungbuk National University, 1 Chungdearo, Seowon-gu, Cheongju 28644, Republic of Korea. E-mail address: hsjeong@chungbuk.ac.kr (H.S. Jeong).

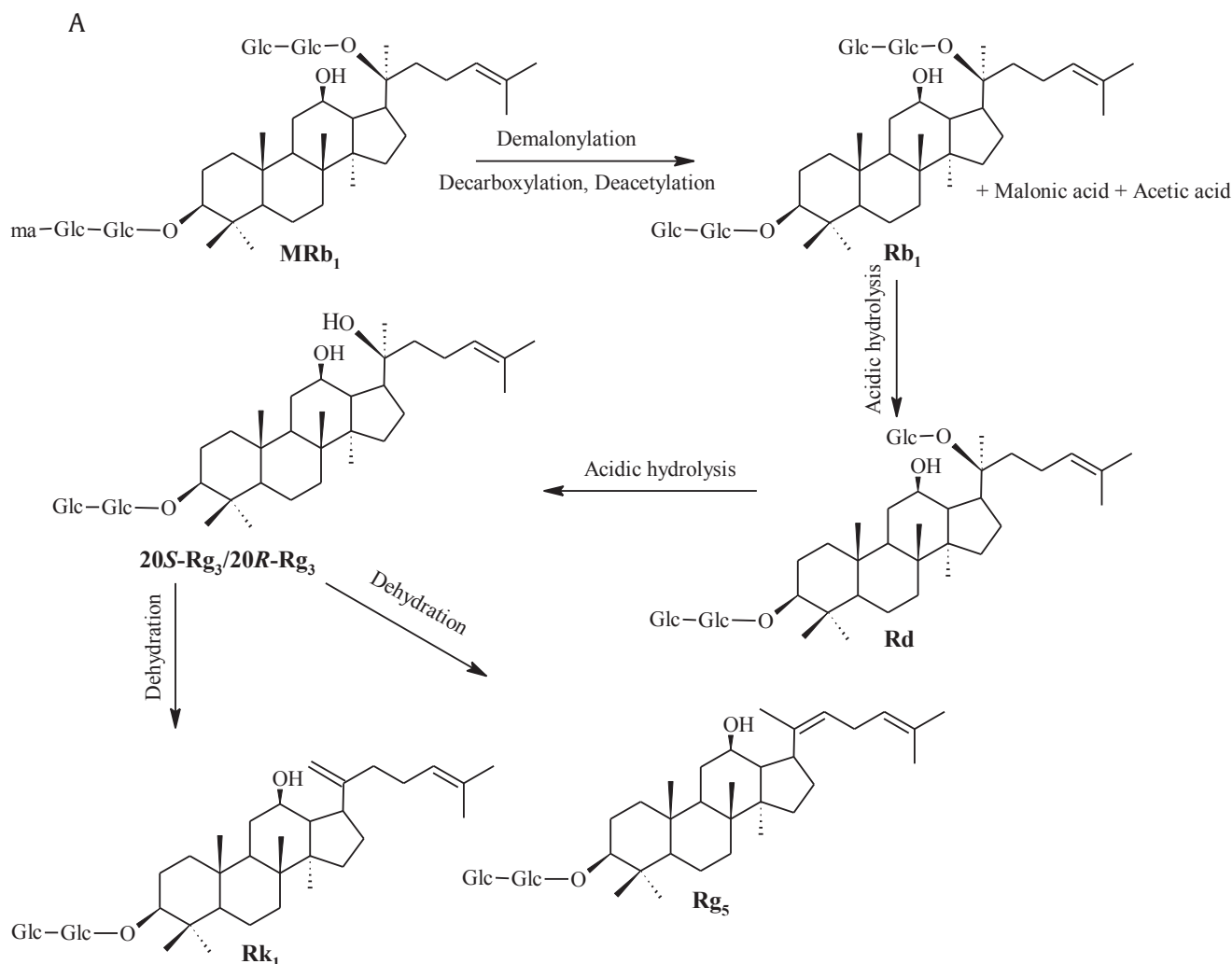


Fig. 1. Transformation pathways during the steaming process of *Panax ginseng*. (A) Malonyl ginsenoside Rb1. (B) ginsenoside Re. Note. From: "Remarkable impact of acidic ginsenoside and organic acids on ginsenoside transformation from fresh ginseng to red ginseng," by Z. Liu Z, J. Xia, C.Z. Wang, J.Q. Zhang, C.C. Ruan, G.Z. Sun, C.S. Yuan, 2016, *J Agric Food Chem*, 64, p. 5389–99. Copyright 20XX, Name of the Copyright Holder. With permission.

ginseng [14], changes in ginsenoside composition by roasting [2], and gamma irradiation of the conversion of ginsenoside Rb₁ to Rg₃ [15] were previously conducted. Accordingly, the primary factors that affect ginsenoside conversion include temperature, heating time, extraction solvent, and stability of ginsenosides. However, a clear explanation for the major factors affecting ginsenoside conversion in ginseng and crude saponin upon heat treatment remains to be elucidated.

Therefore, this study was performed to compare the chemical components of raw ginseng and crude saponin upon heat treatment, and to determine the influence of organic acids and heat treatment on ginsenoside conversion.

2. Materials and methods

2.1. Materials

Four-year-old ginseng was purchased from Ginseng Nonghyup (Jeungpyeong, Korea) in 2013. Ginsenoside standards [Rg₁, Re, Rf, Rb₁, Rc, Rg₂(S), Rh₁, Rg₂(R), Rb₂, Rb₃, F₁, Rd, Rg₆, F₂, F₄, Rk₃, Rh₄, Rg₃(S), Rg₃(R), Rk₁, Rg₅, and Rh₂] were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, Sichuan, China). HPLC-

grade water and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals used were of reagent grade.

2.2. Preparation of raw ginseng

Fresh ginseng was rinsed with tap water and frozen at -18°C for 24 h, and subsequently dried using a freeze dryer (Ilshin Biobase FD5508, Kyunggi-do, Korea). The dried ginseng was ground using a hammer mill (Microhammer cutter mill type-3; Culatti AG, Zurich, Switzerland). The resulting ginseng powder was analyzed.

2.3. Extraction of crude saponin

The extraction of crude saponin was performed according to the method reported by Hwang et al. [16]. Forty milliliters of an 80% (v/v) ethanol/water solution was added to flasks containing ginseng; the flasks were sonicated at 40°C for 30 min using an ultrasonicator (WUC-D22H; Daihan Scientific, Wonju-si, South Korea). Three replicate extracts were combined and evaporated using an evaporator (N-1000; Eyela, Tokyo, Japan) at 40°C . The resulting residue was dissolved in 40 mL distilled water and defatted with diethyl ether in a separatory funnel. The defatted aqueous layer was

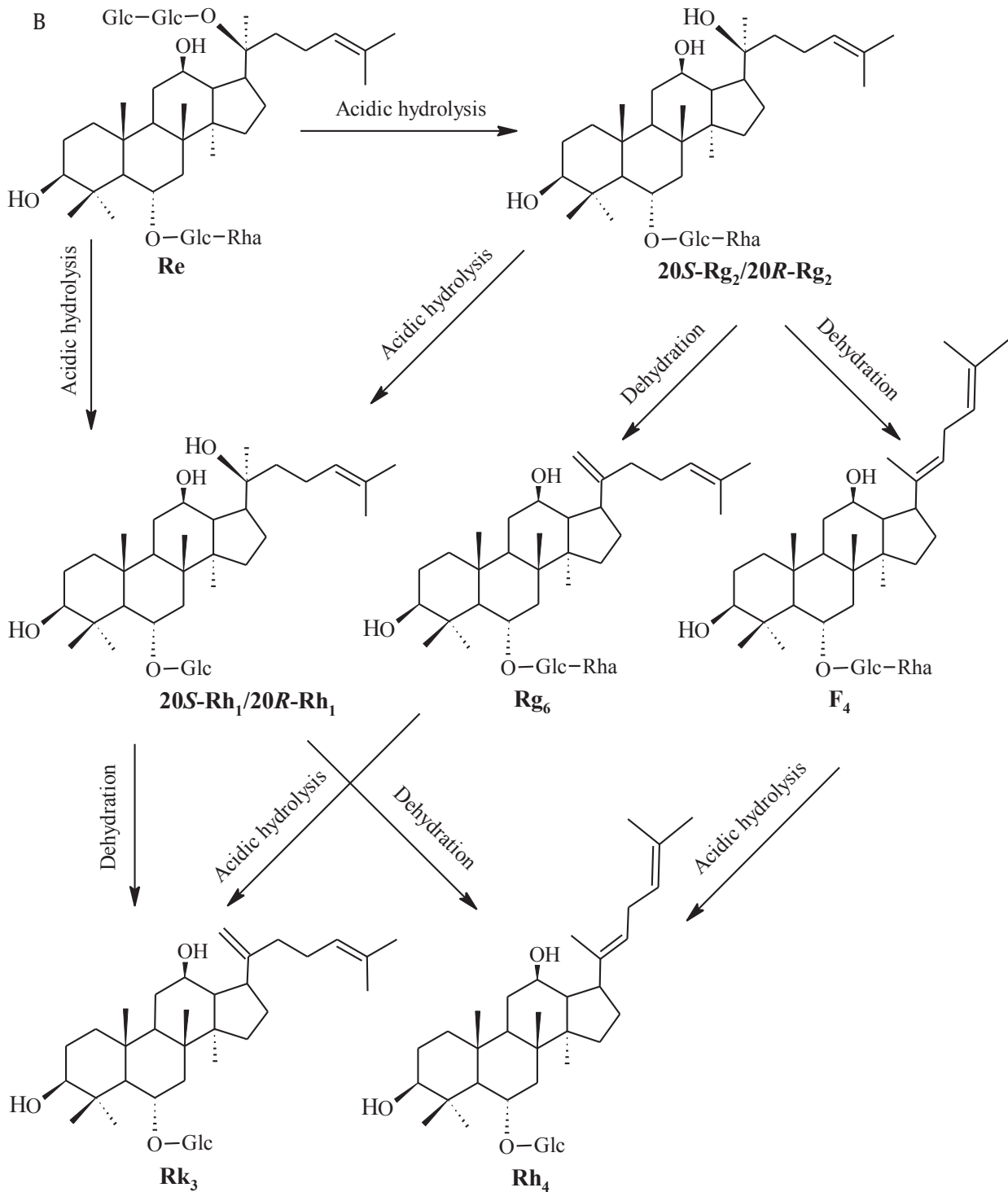


Fig. 1. (continued).

extracted three times with 40 mL water-saturated *n*-butyl alcohol. The *n*-butanol layer was evaporated at 50°C, and the resulting residue was dissolved in distilled water. The dissolved extract was dried using a freeze dryer (FD5508; Ilshin BioBase, Yangju, Korea), and the dried extract was subjected to analysis. The crude saponin content (mg/g ginseng) and ginsenoside composition were determined using the final extract.

2.4. Chemical components of raw ginseng and crude saponin

2.4.1. Ginsenoside composition

The ginsenoside composition was determined according to the method described by Hwang et al. [16]. Crude saponin was dissolved in 4 mL methanol and filtered through a 0.2- μ m membrane filter. The malonyl ginsenoside composition was analyzed

according to a modified version of the method reported by Du et al. [17]. Briefly, the analysis involved the extracted solution of neutral ginsenosides, hydrolysis of the extract to convert the malonyl ginsenosides to the corresponding neutral ginsenosides, analysis of the hydrolyzed extract to determine the total neutral ginsenosides, and calculation of the malonyl ginsenosides using the differential increases in the relevant neutral ginsenosides. The ginsenoside composition was analyzed using HPLC with a UV-visible detector (HPLC system: ACME 9000 system, Younglin, Anyang, South Korea; column: Mightysil RP-18 GP, 250 × 4.6 mm, 5µm i.d., Kanto Chemical Co., Tokyo, Japan). The mobile phase of the analytical system consisted of acetonitrile (A) and water (B), using the following gradient: 0 min (18% A), 0–42 min (24% A), 42–46 min (29% A), 46–75 min (40% A), 75–100 min (65% A), 100–135 min (85% A), and 135–150 min (85% A). The flow rate, detection wavelength, and injection volume were set at 0.6 mL/min, 203 nm, and 20 µL, respectively.

2.4.2. Total phenolic content

The total phenolic contents of crude saponin and ginseng were determined using the Folic–Ciocalteu method [18]. In a 2-mL tube, 0.1 mL of the sample was mixed with 0.4 mL of 2% Na₂CO₃ and 0.1 mL of 50% Folin–Ciocalteu phenol reagent (Sigma Chemical). After exactly 30 min, the absorbance was measured at 750 nm using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan), and the total phenolic content was calculated from a calibration curve ($R^2 = 0.9995$) using gallic acid as the standard. All samples were analyzed in triplicate.

2.5. Heat treatment of raw ginseng, crude saponin, and ginsenoside Rb₁

Heated ginseng and crude saponin were prepared according to the following procedure: equal amounts of ginseng and crude saponin powder (0.5 g) were first weighed in their respective capped glass vials, prior to adding 3.0 mL distilled water. In order to induce more changes than the general processing temperatures (below 100°C), these samples were then heated in an oil bath with a temperature controller (Changshin Science Co., Seoul, Korea) at 130°C for 1–5 h.

Ginsenoside Rb₁ standard was prepared according to the following procedure: 100 µL of a 0.5mM ginsenoside Rb₁ solution with 2mM citric, malic, and succinic acids was placed in a 100-µL microcapillary glass pipet (Kimble Chase Life Science and Research Products LLC., Rockwood, TN, USA), and the pipet was sealed using a torch lamp; the pipets containing the ginsenoside solutions were used as the samples. The pipets containing the ginsenoside solutions were heated in an oil bath with a temperature controller (Changshin Science Co., Seoul, Korea) at 130°C for 1 h. The ginsenoside composition of these samples was analyzed.

2.6. Organic acid contents of ginseng and crude saponin upon heat treatment

The organic acid content of the samples was analyzed [19]. Five grams of raw and heated ginseng was extracted with 40 mL of distilled water using a sonicator at 40°C for 1 h; crude and heated crude saponin samples were diluted with water, and filtered using filter paper (Whatman, No. 4) and a 0.45-µm membrane syringe filter (Nylon, Whatman). The organic acid content of the filtrates was determined. The analysis was performed using HPLC with a UV detector (HPLC system: ACME 9000 system, Younglin, Anyang, South Korea; column: Aminex ion exclusion HPX-87H, 7.8 × 300 mm, Bio-Rad Laboratories, Hercules, CA, USA). A flow rate of 1.0 mL/min was used for the isocratic elution, with a 0.008N H₂SO₄

solution as the mobile phase. Detection was performed with a UV detector at 215 nm. All samples were analyzed in triplicate.

3. Results and discussion

3.1. Chemical components of raw ginseng and crude saponin

The chemical components of raw ginseng and crude saponin are shown in Table 1. The total saponin, total phenolic contents, and total organic acid contents were 3.48%, 0.32%, and 5.55% in raw ginseng, and 64.14%, 1.21%, and 0.00% in crude saponin, respectively; organic acids were not detected in crude saponin.

The ginsenoside composition of raw ginseng is shown in Fig. 2; malonyl ginsenosides and neutral ginsenosides comprised 51.24% and 48.76% of the total ginsenoside content (34.78 mg/g), respectively. The malonyl ginsenoside Rb₁ (m-Rb₁), m-Rc, m-Rb₂, m-Rb₃, m-Rd, Rg₁, Re, Rf, Rb₁, Rc, Rg₂(S), Rb₂, Rb₃, and Rd contents in raw ginseng were 9.27 mg/g, 3.98 mg/g, 2.47 mg/g, 0.45 mg/g, 1.65 mg/g, 3.29 mg/g, 4.31 mg/g, 1.21 mg/g, 4.56 mg/g, 0.63 mg/g, 1.32 mg/g, 0.88 mg/g, 0.17 mg/g, and 0.59 mg/g, respectively. Malonyl ginsenosides, which have a malonyl group at C-3 in protopanaxadiol, are unstable at high temperatures, and are readily converted into neutral ginsenosides through demalonylation upon thermal treatment [20]. Therefore, the neutral ginsenoside content may increase because of the demalonylation of malonyl ginsenosides.

3.2. Ginsenoside composition upon heat treatment

Changes in the ginsenoside composition of raw ginseng and crude saponin due to heat treatment at 130°C are shown in Fig. 3. As the heating time increased, the major ginsenoside Rg₁, Re, Rb₁, Rc, Rh₁, Rg₂, Rb₂, and Rb₃ content decreased, and whereas those of minor ginsenosides Rg₆, F₂, F₄, Rk₃, Rh₄, Rg₃(S), Rg₃(R), Rk₁, and Rg₅ showed the opposite trend in ginseng and crude saponin upon heat treatment. It was reported that glycosylated ginsenosides were converted to deglycosylated and dehydrated ginsenosides at C-20 in the presence of organic acids [12,21]. Ginseng and crude saponin exhibited similar changes in regard to ginsenoside composition upon heat treatment. Raw ginseng and crude saponin contained 3.29 mg/g, 4.31 mg/g, 1.21 mg/g, 4.56 mg/g, 0.63 mg/g, 1.32 mg/g, 0.88 mg/g, 0.17 mg/g, and 0.59 mg/g ginsenoside Rg₁, Re, Rf, Rb₁, Rc, Rg₂(S), Rb₂, Rb₃, and Rd in raw ginseng, and 66.73 mg/g, 54.38 mg/g, 22.05 mg/g, 63.04 mg/g, 852 mg/g, 21.96 mg/g, 17.74 mg/g, 2.78 mg/g, and 4.36 mg/g in crude saponin, respectively. The major ginsenosides (Rb₁, Re, and Rg₁) in raw ginseng and crude saponin decreased upon heat treatment, and those in raw ginseng decreased faster than those in crude saponin. In raw ginseng, major ginsenosides (Rb₁, Re, and Rg₁), which accounted for more than 70% of total ginsenosides, were almost completely converted to minor ginsenosides [Rg₆, F₄, Rk₃, Rh₄, Rg₃(S), Rg₃(R), Rk₁, and Rg₅] in 1 h at 130°C, and the contents of Rg₆, Rk₃, Rh₄, Rg₃(S), Rg₃(R), Rk₁, and Rg₅ increased within 3 h, and the contents of Rg₆, Rk₃, and Rh₄ increased within 5 h upon heat treatment. In crude saponin,

Table 1
Chemical components of raw ginseng and crude saponin

Samples	Chemical components (%)		
	Total saponin	Total phenolic content	Total organic acid content
Raw ginseng	3.48 ± 0.08	0.32 ± 0.03	5.55 ± 0.39
Crude saponin ¹⁾	64.14 ± 0.15	1.21 ± 0.04	ND ²⁾

¹⁾ Yield of crude saponin from raw ginseng: 5.50 ± 0.28%

²⁾ Not detected

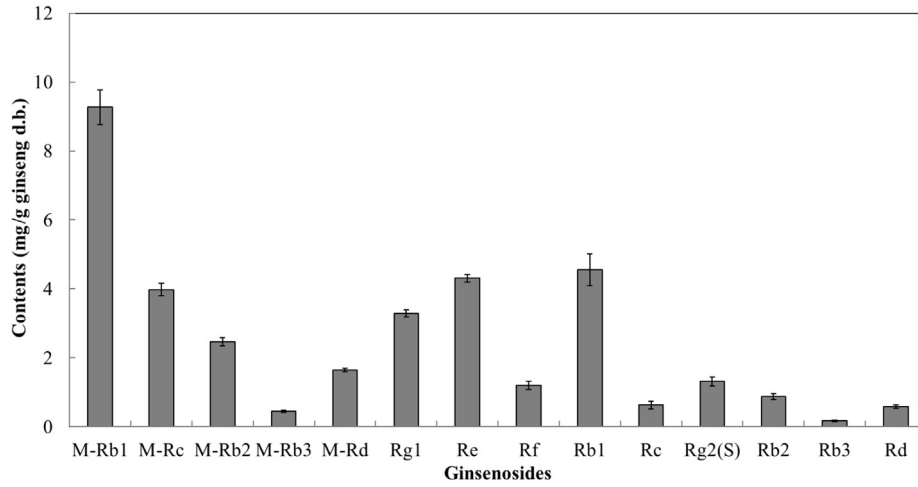


Fig. 2. Ginsenoside composition of raw ginseng without heat treatment.

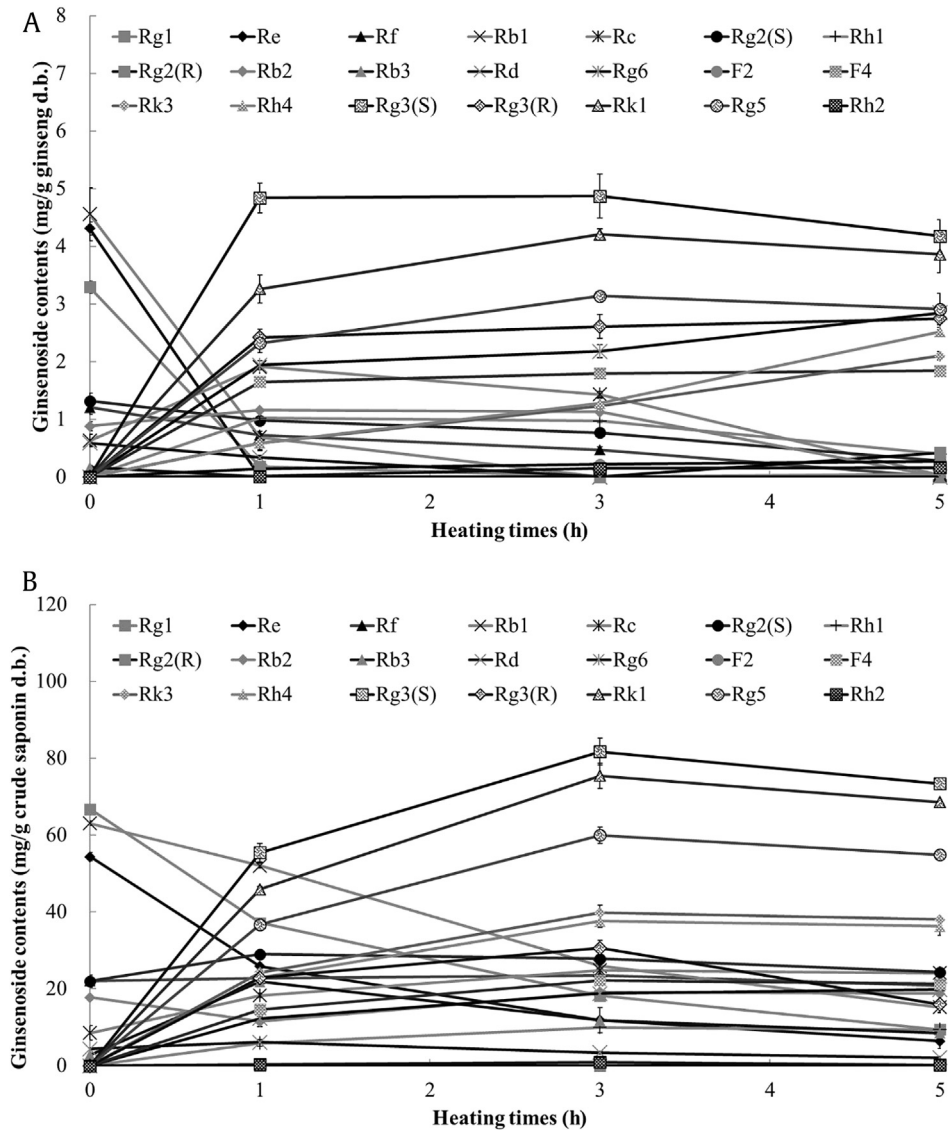


Fig. 3. Changes in ginsenoside composition of ginseng and crude saponin isolated from ginseng during heat treatment at 130°C. (A) Sample A (ginseng). (B) Sample B (crude saponin isolated from raw ginseng).

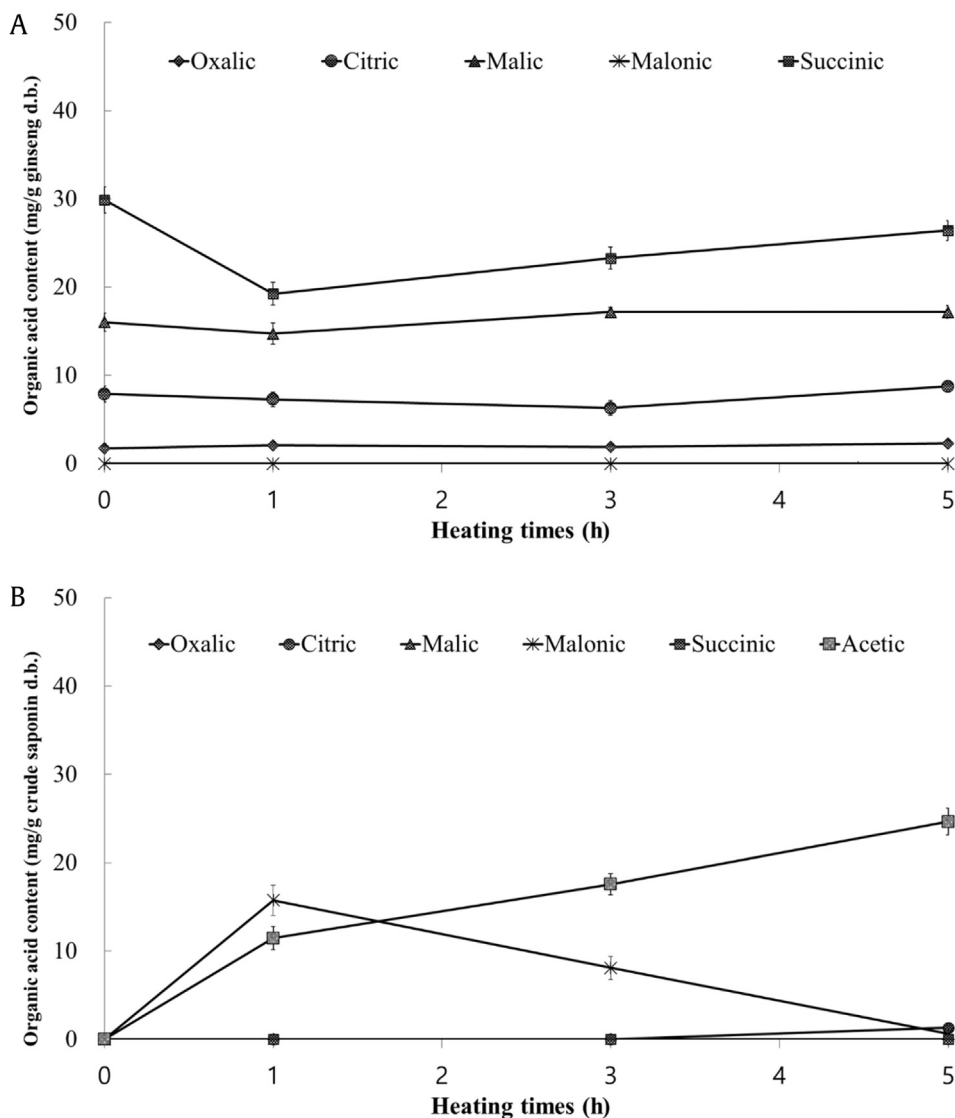


Fig. 4. Changes in organic acid composition of ginseng and crude saponin isolated from ginseng during heat treatment at 130°C. (A) Sample A (ginseng). (B) Sample B (crude saponin isolated from ginseng).

ginsenoside Rb₁, Re, and Rg₁ slowly decreased in comparison with ginseng upon heat treatment, and types of converted ginsenosides from ginsenoside Rb₁, Re, and Rg₁ were similar to those of raw ginseng with heat treatment. The organic acids in ginseng and crude saponin were postulated to cause the differences in the conversion ratios between ginseng and crude saponin. The changes in ginsenoside composition in ginseng upon heat treatment were in accord with previous results [13]. Specifically, it was reported that major ginsenosides such as Rg₁, Re, Rb₁, Rc, Rb₂, and Rd decreased, and Rg₃ increased upon repeated steaming treatments [22]. Nam et al. [11] reported that the ginsenoside Rb₁, Rb₂, Rc, Rd, Re, Rf, and Rg₁ contents decreased, and ginsenoside Rg₃(S), Rg₃(R), and Rk₁ contents in ginseng increased with increasing steaming times.

3.3. Organic acid content following heat treatment

To determine the differences in conversion ratio between ginseng and crude saponin, the organic acid contents in ginseng and crude saponin were measured following various heating times (Fig. 4). The total organic acid content was 5.55% in raw ginseng,

and organic acids were not detected in unheated crude saponin. However, organic acids were found in crude saponin after heat treatment. In raw ginseng, 29.88 mg/g, 16.03 mg/g, 7.90 mg/g, and 1.73 mg/g succinic acid, malic acid, citric acid, and oxalic acids were found, respectively. As the heating time was increased from 1 h to 5 h, the succinic acid, malic acid, and citric acid contents slightly increased from 19.26 mg/g, 14.73 mg/g, and 7.27 mg/g to 26.42 mg/g, 17.20 mg/g, and 8.75 mg/g, respectively, in heated ginseng. In heated crude saponin, malonic acid, acetic acid, and citric acid were found, and malonic acid derived from malonyl ginsenosides was almost completely converted to acetic acid (24.66 mg/g) at 130°C in 5 h. It was thought that the effect of organic acids from raw ginseng on ginsenoside conversion in heated ginseng was higher than malonic acid and acetic acid from malonyl ginsenosides because the total content of organic acids (5.55% in raw ginseng) was relatively higher than acetic acids (approximately 0.14% in heated ginseng) derived from malonic acid of malonyl ginsenosides in raw ginseng or heated ginseng.

Liu et al. [9] reported that low-molecular-weight organic acids (LMWOAs) and acidic ginsenosides (malonyl ginsenosides) affected

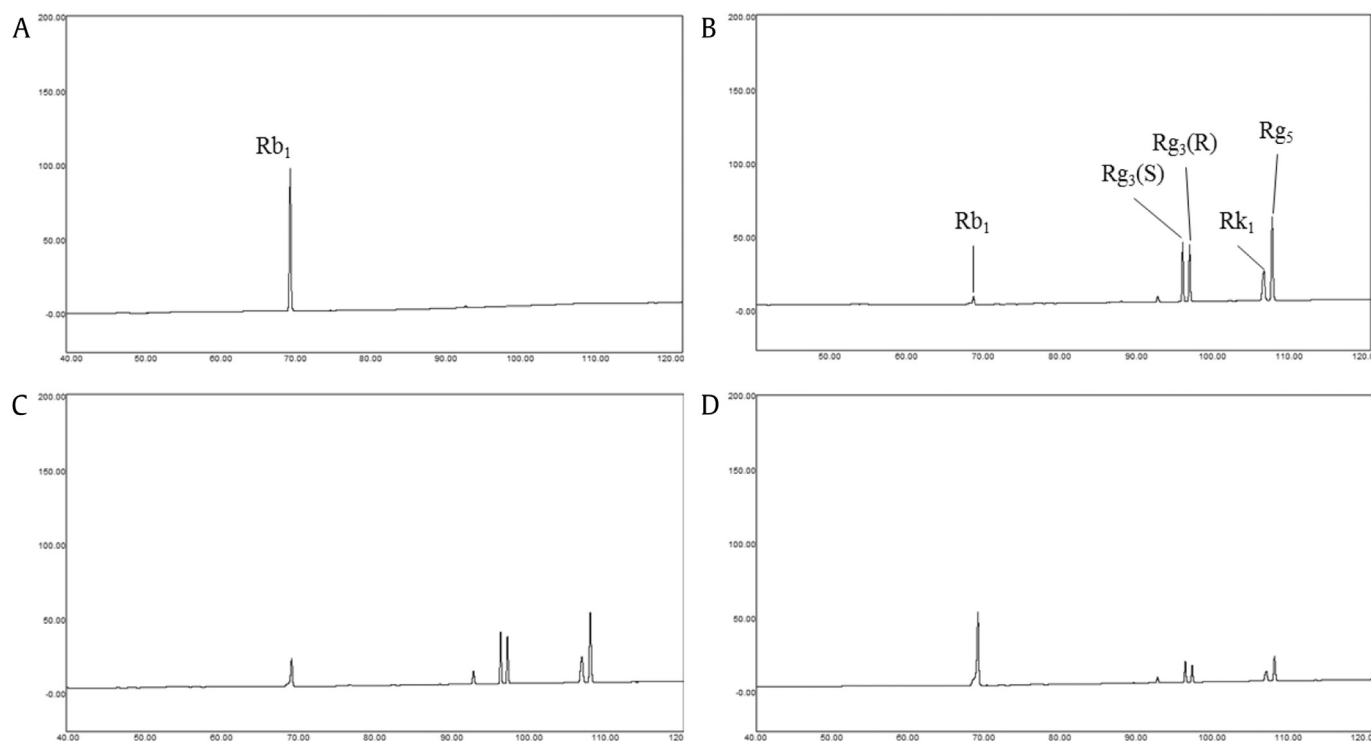


Fig. 5. Changes in chromatograms of ginsenoside Rb₁ with different organic acids during heat treatment at 130°C. (A) Sample A (ginsenoside Rb₁ with water). (B) Sample B (ginsenoside Rb₁ with 2mM citric acid solution). (C) Sample C (ginsenoside Rb₁ with 2mM malic acid solution). (D) Sample D (ginsenoside Rb₁ with 2mM succinic acid solution).

ginsenoside transformation; the major LMWOAs were succinic acid, citric acid, acetic acid, malic acid, and lactic acid in fresh ginseng and succinic acid, acetic acid, citric acid, malic acid, lactic acid, and malonic acid in ginseng heated at 120°C. Jeong et al. [23] reported that fresh and white ginseng contained oxalic acid, malonic acid, succinic acid, malic acid, and citric acid, and major organic acids in ginseng included citric acid and malic acid. Malonic acid is derived from malonyl ginsenosides (m-Rb₁, m-Rb₂, m-Rb₃, m-Rc, and m-Rd), which have malonic acid functionalities at C-3 [20], and acetic acid, which increased with increased heating times, is derived from malonic acid originated from malonyl ginsenosides through decarboxylation during heat treatment [24]. The increase in organic acid content in ginseng upon heat treatment was primarily attributable to a change from free sugars to organic acids through tautomerization, retro aldol reaction, and dehydration by heat treatment [25–27].

3.4. Effects of organic acids on ginsenoside Rb₁ conversion

Ginsenoside Rb₁ was highly stable in distilled water in the absence of organic acids at high temperatures. However, when ginsenoside Rb₁ was treated with organic acids at 130°C for 1 h, it was readily converted into deglycosylated and dehydrated ginsenosides at C-20 in water (Fig. 5). This result is in good agreement with previous study comparing the effect between acidic ginsenosides and organic acids on ginsenoside transformation, and Liu et al. [9] reported that the degradation of ginsenoside Rb₁ readily occurred at acidic conditions (pH 5.0–6.0) during the steaming process; however, the degradation was not observed under neutral pH conditions. Ginsenoside Rb₁ was converted into Rg₃(S), Rg₃(R), Rk₁, and Rg₅. Differences in the conversion ratio of ginsenoside Rb₁ with various organic acids (citric acid, malic acid, and succinic acid) were found; when citric acid was added, the conversion ratio of ginsenoside Rb₁ treated with citric acid was higher than that of ginsenosides treated with other organic acids because of the

difference in the pK_a value, which was in accord with previous research [28]. Notably, the pK_a values of citric acid, malic acid, and succinic acid are 3.14, 3.40, and 4.20 in water at 25°C, respectively, and organic acids with lower pK_a values are more acidic and have more effect on hydrolysis [29,30].

In conclusion, the ginsenoside composition in ginseng and crude saponin was altered upon heat treatment, and ginsenoside Rb₁ was very stable in water in the absence of organic acids at high temperatures, whereas in the presence of organic acids, this standard was unstable. Organic acids such as succinic acid, malic acid, citric acid, oxalic acid, and malonic acid derived from malonyl ginsenosides considerably affected the ginsenoside conversion in ginseng. Therefore, organic acids, ginsenoside content, and processing conditions should be considered important factors that affect the quality of ginseng products.

References

- [1] Takagi K, Saito H, Nabata H. Pharmacological studies of *Panax ginseng* root: estimation of pharmacological actions of *Panax ginseng* root. *Jpn J Pharmacol* 1972;22:245–59.
- [2] Park MH, Park CK, Lee KS, Kim KC. Changes of ginsenoside in ginseng marc by roasting process. *Korean J Ginseng Sci* 1996;20:184–7.
- [3] Matsuura H, Kasai R, Tanaka O, Saruwatari Y, Kunihiro K, Fuwa T. Further studies on the dammarane-saponins of ginseng roots. *Chem Pharm Bull* 1984;32:1188–92.
- [4] De Smet PAGM. Herbal remedies. *N Engl J Med* 2002;347:2046–56.
- [5] Kim JH, Ahn SC, Choi SW, Hur NY, Kim BY, Baik MY. Changes in effective components of ginseng by puffing. *J Appl Biol Chem* 2008;51:188–93.
- [6] An YE, Ahn SC, Yang DC, Park SJ, Kim BY, Baik MY. Chemical conversion of ginsenosides in puffed red ginseng. *LWT* 2011;44:370–4.
- [7] Lee GS, Nam KY, Choi JE. Ginsenoside composition and quality characteristics of red ginseng extracts prepared with different extracting methods. *J Med Crop Sci* 2013;21:276–81.
- [8] Han BH, Park MH, Han YN, Woo LK, Sankawa U, Tanaka O. Degradation of ginseng saponins under mild acidic conditions. *Planta Med* 1982;44:146–9.
- [9] Liu Z, Xia J, Wang CZ, Zhang JQ, Ruan CC, Sun GZ, Yuan CS. Remarkable impact of acidic ginsenoside and organic acids on ginsenoside transformation from fresh ginseng to red ginseng. *J Agric Food Chem* 2016;64:5389–99.

- [10] Hong HD, Kim YC, Rho J, Kim KT, Lee YC. Changes on physicochemical properties of *Panax ginseng* C. A. Meyer during repeated steaming process. *J Ginseng Res* 2007;31:222–9.
- [11] Nam KY, Lee NR, Moon BD, Song GY, Shin HS, Choi JE. Changes of ginsenosides and color from black ginseng prepared by steaming–drying cycles. *Korean J Med Crop Sci* 2012;20:27–35.
- [12] Choi KH, Kwak YS, Rhee MH, Hwang MS, Kim SC, Park CK, Han GH, Song KB. Effects of pH and high temperature treatment on the changes of major ginsenosides composition in Korean red ginseng water extract. *J Ginseng Res* 2008;32:127–34.
- [13] Choi WY, Lee CG, Seo YC, Song CH, Lim HW, Lee HY. Effects of high pressure and steaming extraction processes on ginsenosides Rg3 and Rh2 contents of cultured–root in wild ginseng (*Panax ginseng* C. A. Meyer). *Korean J Med Crop Sci* 2012;20:270–6.
- [14] Chen R, Meng F, Zhang S, Liu Z. Effects of ultrahigh pressure extractions on yields and antioxidant activity of ginsenoside from ginseng. *Sep Purif Technol* 2009;66:340–6.
- [15] Kim JH, Kwon SK, Sung NY, Jung PM, Choi JI, Kim JK, Sharma AK, Lee JW. Effect of gamma irradiation on the conversion of ginsenoside Rb1 to Rg3. *Radiat Phys Chem* 2012;81:1128–31.
- [16] Hwang CR, Lee SH, Jang GY, Hwang IG, Kim HY, Woo KS, Lee J, Jeong HS. Changes in ginsenoside compositions and antioxidant activities of hydroponic–cultured ginseng roots and leaves with heating temperature. *J Ginseng Res* 2014;38:180–6.
- [17] Du XW, Wills RBH, Stuart DL. Changes in neutral and malonyl ginsenosides in American ginseng (*Panax quinquefolium*) during drying, storage and ethanolic extraction. *Food Chem* 2004;86:155–9.
- [18] Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem* 2006;95:200–4.
- [19] Yoon YJ, Kim NY, Rhee YK, Han MJ. Quality characteristics and biological activities of traditionally fermented ginseng wine. *Food Sci Biotechnol* 2007;16:198–204.
- [20] Qi L-W, Wang C-Z, Yuan C-S. American ginseng: potential structure–function relationship in cancer chemoprevention. *Biochem Pharmacol* 2010;80:947–54.
- [21] Choi JH, Kim DH, Sung HS, Kim WJ, Oh SK. Kinetic studies on the thermal degradation of ginsenosides in ginseng extract. *Korean J Food Sci Technol* 1982;14:197–202.
- [22] Kim SN, Kang SJ. Effects of black ginseng (9 times–steaming ginseng) on hypoglycemic action and changes in the composition of ginsenosides on the steaming process. *Korean J Food Sci Technol* 2009;41:77–81.
- [23] Jeong SI, Lee YG, Kim CS, Lee S. Property and inhibitions of the hydrolysis of ginseng saponins by organic acids neutralization in ginseng extract preparations. *Korean J Med Crop Sci* 1998;6:305–10.
- [24] Darensbourg DJ, Holtcamp MW, Khandelwal B, Reibenspies JH. Intramolecular and intermolecular hydrogen bonding in triphenylphosphine derivatives of copper(I) carboxylates, $(\text{Ph}_3\text{P})_2\text{CuO}_2\text{C}(\text{CH}_2)_n\text{COOH}$. Role of copper(I) in the decarboxylation of malonic acid and its derivatives. *Inorg Chem* 1994;33:531–7.
- [25] Woo KS, Hwang IG, Kim HY, Hang KI, Lee JS, Kang TS, Jeong HS. Thermal degradation characteristics and antioxidant activity of fructose solution with heating temperature and time. *J Med Food* 2011;14:167–72.
- [26] Hwang CR. Effects of heat treatment on chemical components, biological activities and ginsenoside contents in roots and leaves of hydroponic–cultured ginseng. Chungbuk, Korea: Chungbuk National University; 2011.
- [27] Aida TM, Sato Y, Watanabe M, Tajima K, Nonaka T, Hattori H, Arai K. Dehydration of d-glucose in high temperature water at pressure up to 80 MPa. *J Supercrit Fluid* 2007;40:381–8.
- [28] Kim MH, Lee YC, Choi SY, Cho CW, Rho J, Lee KW. The changes of ginsenoside patterns in red ginseng processed by organic acid impregnation pretreatment. *J Ginseng Res* 2011;35:497–503.
- [29] Bruice PY. Organic chemistry. 5th ed. USA: Pearson Education; 2014.
- [30] Rived F, Roses M, Bosch E. Dissociation constants of neutral and charged acids in methyl alcohol. The acid strength resolution. *Anal Chim Acta* 1998;374:309–24.