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Ginsenoside Changes in Red Ginseng Manufactured by Acid Impregnation Treatment

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To enhance the functionalities of ginseng, an acid impregnation pre-treatment was applied during red ginseng processing. Acetic, ascorbic, citric, malic, lactic, and oxalic acid were used for the acid impregnation treatment, and total and crude saponin concentrations and ginsenoside patterns were evaluated. Total and crude saponin contents of red ginseng pre-treated by acetic, ascorbic, and citric acid were similar to those of red ginseng without pre-treatment, whereas lactic, malic, and oxalic acid pre-treatment caused a reduction of total and crude saponin in red ginseng. From the high performance liquid chromatography analysis of ginsenosides, increased Rg_3 density was shown in red ginseng pre-treated by acetic, ascorbic, and citric acid impregnation. In the case of lactic, malic, and oxalic acid pre-treatment, increased Rg_1 density was observed in red ginseng. Increased Rg_1 and Rg_3 contents due to acid impregnation during red ginseng processing may contribute to improving bioactive functionalities of red ginseng.

Keywords: Ginsenoside, Red ginseng, Acid impregnation, Rg_3

INTRODUCTION

Korean ginseng (the root of *Panax ginseng* C. A. Meyer) has been known for several thousand years to have superior pharmacological effects in the human body, and it is the most representative herbal medicine in East Asian countries including Korea, China, and Japan.

In modern times, Korean ginseng has been spotlighted again as a natural health food following scientific assessments of various pharmacological effects of Korean ginseng and its major components. The demand for ginseng has risen dramatically and has been accelerated by economic development and improvements in living conditions [1-3]. Various chemical components derived from Korean ginseng and their pharmacological effects have been assessed through modern scientific research. Among various components of Korean ginseng, ginsenosides are known to be major beneficial components [4,5]. Forty species of ginsenosides have been found. Ginsenosides have a broad effect on the central nervous system, endocrine system, immune system, and metabolism, resulting in control of body dysfunction and normalization of physiological dysfunction [6-9]. Among various ginsenosides, ginsenoside Rh_2 and ginsenoside Rg_3 have received considerable attention recently. Ginsenoside Rh_2 has strong inhibitory effects on the proliferation of several cancer cells and the ability to change some cancer cells to normal cells by promoting differentiation of cancer cells. Ginsenoside Rg_3 has inhibitory effects on cancer metastasis, tolerance for anti-cancer agents, platelet aggregation, thrombosis and liver dam-

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age, and it enhances relaxation of blood vessels [10,11]. However, these compounds are found in trace amounts in Korean ginseng. Increased concentrations of ginsenosides in Korean ginseng could add value to this commodity. It may be possible to increase the market for Korean ginseng, as an increase in Korean ginseng's functionality would increase the inhibitory effects on cancer metastasis and tolerance for anti-cancer agents [12]. Therefore, the present study investigated several acid treatments of Korean red ginseng intended to increase specific ginsenoside functions. The manufacture of specific ginseng products containing increased Rg_3 or other specific ginsenosides through deglycosylation, ultra high pressure, or heat treatments has been investigated. But the efficiency of these processes is insufficient for industry application. Also, previous studies focused not on ginseng itself, but on ginseng [13,14].
The present study was conducted to investigate selective physiological activity of red ginseng through the application of various organic acid treatments to fresh ginseng and assessment of resulting changes in ginsenoside contents.

MATERIALS AND METHODS

Materials

Fresh ginseng root (4 or 5 year) was purchased from Anseong Korean Ginseng Nonghyup (Anseong, Korea). The roots were stored at - 2°C to 0°C before use. For the impregnation of organic acids, two clean, washed, fresh ginseng roots (approximately 140 g each) were placed in a large volumetric flask and 500 mL of 1M organic acid was added to allow the ginseng to be submerged sufficiently. This solution was placed under vacuum for 30 min. The organic acids were acetic acid, citric acid, lactic acid, oxalic acid, malic acid, and ascorbic acid (all obtained from Sigma, St Louis, MO, USA).

Manufacture of Korean red ginseng by organic acids

Korean red ginseng was manufactured by the method of Chang *et al*. [15] from fresh ginseng treated by organic acid impregnation. Ginseng impregnated with organic acid was steamed in an autoclave (MG-6845; Mega Science, Seoul, Korea) at 97±1°C for 2.5 h and cooled at room temperature. The resulting ginseng was dried at 70°C for 24 h and at 50°C for 72 h in a forced convection oven (WFO-601SD; Eyela, Tokyo, Japan). Water contents of the dried ginseng were below 12%. More than six roots of the red ginseng manufactured from each organic acid treatment were pooled for use.

Measurement of saponin contents

Pulverized red ginseng was passed by 60 mesh. One hundred milliliters of 80% aqueous methanol were added to two grams of powder sample and extracted at 80°C for 3 hours twice using a reflux condenser. Then, the collected extracts were concentrated with a rotary vacuum evaporator. The resulting concentrates were dissolved in 20 mL of distilled water and used as saponin extracts. Saponin extracts were washed with 20 mL of ethyl ether using separatory funnels and extracted three times using 20 mL of water-saturated *n*-butanol. The resulting extracts were washed twice with 60 mL of distilled water. The *n*-butanol layer was concentrated with a rotary vacuum evaporator, and the weight of the resulting concentrates was measured for saponin contents. The resulting concentrates were dissolved in 2 mL of methanol and filtered using a 0.45-μm syringe filter (Whatman; Maidstone, Kent, UK). The filtrates were used for saponin composition analysis. The amounts of saponin are presented as mean and standard deviation with triplicate analysis.

HPLC analysis of saponin composition

Chromatographic analysis of saponin composition was performed using high performance liquid chromatography (HPLC). Standards of ginsenosides (Chengdu, PR China) were Rg_1 (99.2% purity), Re (98.6%), Rf (98.9%), Rb_1 (98.9%), Rg_2 (98.2%), Rh_1 (98.4%), Rc (98.7%), Rb_2 (98.2%), Rb_3 (98.1%), Rd (98.1%), Rg_3 (99.0%), and Rh_2 (98.2%). Concentration of each ginsenoside standard was 0.1 mg/mL. Separation was carried out using μ-Bondapak C_{18} column (Waters; Milford, MA, USA). The mobile phase consisted of acetonitrile:H_2O (3:7). The flow rate was 1.6 mL/min. The detector was a UV detector (UV-2075 PLUS UV-vis; JASCO, Tokyo, Japan), and absorbance was measured at 203 nm [16].

RESULTS AND DISCUSSION

It is difficult to penetrate acid to ginseng even immersed in an acid solution. Therefore, acid impregnation method used for fruits was also used for fresh ginseng. Acid penetration into ginseng was identified using a specific dye, as seen in Fig. 1. Control (non-organic acid-treated) steamed ginseng was compared to the steamed ginseng treated by several organic acids prior

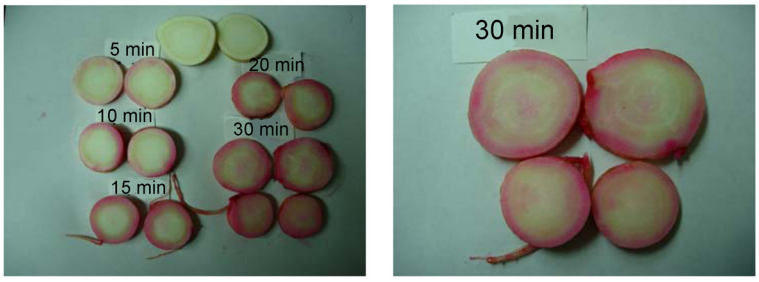

Fig 1. Acid penetration into fresh ginseng observed with dye method. Left panel, 5 to 30 min; Right panel, 30 min.

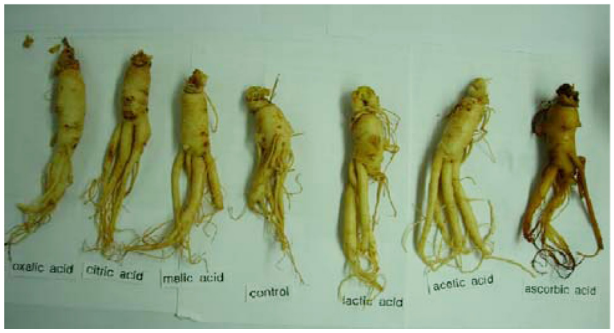

Fig. 2. Steamed ginseng after acid impregnation.

Fig. 3. Red ginseng after acid impregnation pre-treatment.

to dryness (Fig. 2). Control (non-organic acid-treated) steamed ginseng was not different from the steamed ginseng treated by several organic acids based on their appearances, but oxalic acid-treated steamed ginseng and ascorbic acid-treated steamed ginseng had different color bearing dark color during the drying process (Fig. 3). Also, red ginseng produced by this new pre-treatment showed improved flavor compared to currently available red ginsengs through a sensory test. Other organic acid treated red ginseng is needed for a sensory test. If it is used, it should be applied to the development of red ginseng's flavor-improvement technology [17].

The saponin content of control (non-organic acid-treated) red ginsengs was 3.43%. There was little difference between saponin contents in citric acid-treated red ginseng, acetic acid-treated red ginseng, and ascorbic acid-treated red ginseng. However, saponin contents were reduced in malic acid-treated red ginseng, oxalic acid-treated red ginseng, and lactic acid-treated red ginseng (Table 1). It is thought that water-soluble saponin in malic acid-treated red ginseng may be eluted during acid impregnation. The pH of organic acid solution was increased by approximately 0.1 after impregnation in each organic acid treatment. One possible explanation is that most of the organic acid solution was penetrated into the ginseng, but water contained in the ginseng was eluted in the organic acid solution, resulting in a pH increase of the organic acid solution. However, pH of the malic acid solution was increased greatly after impregnation. This may be explained by elution of higher amount of water and this elution may be accompanied with elution of water soluble saponins of fresh ginseng into malic acid solution [18].

Table 1. The changes of saponin contents in red ginseng by acid pre-treatment (n=3)

	Crude saponin (%)
Control	3.433 ± 0.00^{b}
Citric acid	3.354 ± 0.06^{b}
Acetic acid	3.404 ± 0.05^{b}
Lactic acid	2.923 ± 0.15^{c}
Oxalic acid	2.604 ± 0.31^{d}
Malic acid	1.995 ± 0.13^{e}
Ascorbic acid	3.983 ± 0.09^{a}

Values are presented as mean±SD.
Mean with different superscripts in same row are significantly different (p<0.05) by Duncan's multiple rage test.

Analysis of the ginsenoside content of red ginsengs produced by different organic acid treatments is presented in Table 2. The various red ginsengs showed different patterns according to pre-treatment organic acids. Rg_3 contents were significantly increased in ascorbic acid- and citric acid-treated red ginseng, especially high

Table 2. The changes of ginsenosides contents in red ginseng by acid pre-treatment (mg/g)

	Control	Citric acid	Acetic acid	Lactic acid	Oxalic acid	Malic acid	Ascorbic acid
Rg1	0.049±0.002	0.027±0.001	0.020±0.001	0.095±0.001	0.145±0.004	0.120±0.003	0.013±0.000
Re	0.030±0.001	0.016±0.001	0.017±0.001	0.049±0.002	0.085±0.002	0.104±0.002	0.013±0.000
Rf	0.052±0.002	0.049±0.003	0.044±0.002	0.051±0.001	0.053±0.001	0.036±0.001	0.042±0.001
Rb1+Rg2+Rh1	0.189±0.004	0.135±0.005	0.151±0.004	0.132±0.004	0.036±0.001	0.038±0.002	0.148±0.004
Rc	0.047±0.001	0.076±0.004	0.043±0.001	0.078±0.002	0.130±0.002	0.105±0.001	0.043±0.001
Rb2	0.052±0.001	0.098±0.002	0.072±0.002	0.082±0.002	0.092±0.001	0.057±0.001	0.047±0.001
Rb3	0.017±0.001	0.029±0.001	0.043±0.001	0.011±0.000	0.015±0.000	0.009±0.000	0.016±0.000
Rd	0.040±0.002	0.049±0.002	0.027±0.000	0.029±0.001	0.032±0.001	0.021±0.001	0.022±0.001
Rg3	0.028±0.001	0.135±0.004	0.031±0.001	0.017±0.000	0.018±0.000	0.020±0.000	0.078±0.002
Rh2	0.002±0.000	0.001±0.000	0.004±0.000	0.002±0.000	0.002±0.000	0.016±0.000	0.004±0.000

Values are presented as mean±SD.

in citric acid-treated red ginseng. Previous researches have shown that Rg_3 exhibits anti-cancer effects and improves blood circulation. Thus, it is believed that ascorbic acid- and citric acid-treated red ginseng will exhibit these positive effects [19,20].

Concentrations of Rg_1 were significantly increased in oxalic acid-treated red ginseng, malic acid-treated red ginseng, and lactic acid-treated red ginseng. Furthermore, concentrations of Re and Rc were increased in oxalic acid-treated red ginseng and malic acid-treated red ginseng. This may arise from the two organic acids' selective elimination actions for glucose, over rhamnose or arabinose, in glycosides of ginsenosides. Concentrations of Rb_1, whose major glycoside is glucose, were found to be decreased in organic acid-treated red ginsengs. These results show selective elimination for specific glycosides of ginsenosides. Therefore, further study is needed to elucidate the mechanism underlying this process.

To enhance the functionalities of ginseng, an acid-impregnation process as a pre-treatment was applied in producing red ginseng. This new pre-treatment could increase specific saponins in red ginseng, resulting in the manufacture of ginsengs presenting various physiological activities. Control (non-organic acid-treated) steamed ginseng was not different from the steamed ginseng treated by most organic acids, but oxalic acid-treated steamed ginseng and ascorbic acid-treated steamed ginseng of black color were different from the control, as they became red ginsengs during the drying process. Saponin concentrations in citric acid-treated red ginseng, acetic acid-treated red ginseng, and ascorbic acid-treated red ginseng were not significantly changed. However, reductions of saponin concentrations were observed in malic acid-treated red ginseng, oxalic acid-treated red ginseng, and lactic acid-treated red ginseng compared with control (non-organic acid-treated) red ginseng. Rg_3 concentration was significantly increased in ascorbic acid-treated red ginseng and citric acid-treated red ginseng, whereas Rg_1 concentration was significantly increased in oxalic acid-treated red ginseng, malic acid-treated red ginseng, and lactic acid-treated red ginseng.

It is expected that the present study may contribute to the development of the ginseng market by encouraging the development of specific processed ginsengs presenting selective physiological activities or new functionalities. This is expected to occur as manufacturing evolves to provide new red ginsengs with increased concentrations of selected saponins, created by adjustments to ginsenoside composition through new pre-treatments.

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