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Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (*Panax ginseng* C.A. Meyer)

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Abstract

Phenolic acids of dried white and red ginsengs were extracted and fractionated into free, esterified, and insoluble-bound forms. The contents of individual phenolic acids in different forms were quantified by gas–liquid chromatography. Identification of all phenolic acids was confirmed by gas chromatography–mass spectrometry and comparison of gas chromatographic retention times. Twelve different phenolic acids as free, esterified, and insoluble-bound forms were identified in Korean ginsengs. Total phenolic acids in white and red ginsengs were 27.2 and 26.8 mg/100 g, respectively. Seven free phenolic acids were identified and their total contents in white and red ginsengs were 4.70 and 4.14 mg/100 g, respectively. *trans*-Ferulic acid was the predominant free phenolic acid, representing 47.9 and 57.7% of total free phenolic acids in white and red ginsengs, respectively. Esterified phenolic acids represented 71.9 and 77.1% of total phenolic acids in white and red ginsengs, respectively. The most predominant esterified phenolic acids were *cis*-ferulic acid and *trans*-ferulic acid. Total insoluble-bound phenolic acid contents in white and red ginsengs were 2.93 and 1.99 mg/100 g, respectively. Ferulic acid (*cis* and *trans* isomers) was also the major insoluble-bound phenolic acids. This paper represents the first report of systematic identification and quantification of phenolic acids in different forms in white and red Korean ginsengs.

Keywords: White ginseng; Red ginseng; Phenolic acid; Isomerization

1. Introduction

Panax ginseng C.A. Meyer (Korean ginseng, mainly produced in Korea and northeast China), is an herbal root and has been used extensively as Chinese medicine and/or functional food for more than 2000 years. The biochemical and pharmacological activities of the ginseng have been known as antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic and antistress, antifatigue, tranquilizing activities, and promotion of DNA, RNA and protein synthesis activities (Abe, Arichi, Hayashi, & Odashima, 1979; Bhattacharya & Mitra, 1991; Deng, Guan, & Kwan, 1990; Han, Park, Han, & Shin, 1984; Sugaya, Yuzurihara, Tsuda, Yasuda, Kajiwara, & Sugaya, 1988). The biochemical and pharmacological studies of Korean ginseng (*P. ginseng* C.A. Meyer) have been mainly concentrated on ginseng saponins (ginsenosides) as effective components. More than 30 different kinds of ginsenosides have so far been isolated and their chemical structures have also been elucidated. However, non-saponin components have recently received a great attention for their antioxidant, anticancer, antidiabetic and immunomodulating activity. Among the non-saponin components in ginseng are the phenolic acids. It has been reported that ginseng extract has strong antioxidant activity on metal-induced lipid peroxidation and human-LDL oxidation, and inhibitory activity on scission of supercoiled DNA strands induced by peroxyl radicals (Han, Park, Woo, Woo, & Han, 1979; Hu & Kitts, 2001; Kitts, Wihewickreme, & Hu, 2000; Zhang et al., 1996). Han, Park, and Han (1981, 1985) isolated and identified maltol, salicylic acid, vanillic acid, and *p*-coumaric acid as principal antioxidant components in Korean ginseng to protect hepatic tissue from ethanol intoxification. Wee, Park, Kim, and Lee (1989a, 1989b) isolated and identified four phenolic acids (salicylic acids, *p*-coumaric acid, gentisic acid and caffeic acid) from antioxidant fractions in Korean ginseng. Wee, Hoe, and Kim (1996) identified two more phenolic acids (*p*-hydroxybenzoic acid and

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1H-indole-2-carboxylic acid) in Korean ginseng. Kim, Wee, and Park (1987) isolated and identified two free phenolic acids (ferulic and vanillic acid) from Korean ginseng. Lim, Ishiguro, and Kubo (1999) identified *p*-coumaric acid as a principal tyrosinase inhibitor from the fresh leaves of *P. ginseng*. Phenolic acids are reportedly present as three different forms of free, esterified and insoluble-bound phenolic acids in plants (Krygier, Sosulski, & Hogge, 1982a, 1982b, 1982c). For the effective utilization of ginseng as a medicinal herb or functional food component, qualitative and quantitative information on the phenolic acids in ginsengs is essential. However, there was only one quantitative report available in the literature on the free phenolic acids in Korean white ginseng (Park, Park, Kim, & Han, 1994). The authors identified salicylic acid, vanillic acid, genistic acids, syringic acid, *p*-coumaric acid, ferulic acid, and caffeic acid in free form in Korean white ginseng. The authors, however, did not isolate and quantify esterified and insoluble-bound phenolic acids in ginseng. Thus, the objectives of the present research were (1) to fractionate the phenolic acids of dried white and red Korean ginsengs into free, esterified, and insoluble-bound forms and (2), after hydrolysis, to determine the contents of individual phenolic acids in different forms by gas–liquid chromatography. Identification of all phenolic acids was confirmed by gas chromatography–mass spectrometry and comparison of gas chromatographic retention times.

2. Materials and methods

2.1. Materials

Salicylic, *p*-hydroxybenzoic, vanillic acid, gentistic, protocatechuic, syringic, *trans*-*p*-coumaric acid, gallic acid, *trans*-ferulic, caffeic, sinapic, and quinic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Six year old fresh ginseng roots (*P. ginseng* C.A. Meyer) were obtained from a ginseng farm in Keumsan, Korea. Forty fresh ginseng roots were cut in half lengthwise. One half was used for preparing dried white ginsengs, and the other half was used for preparing dried red ginseng. Dried white and red ginsengs were prepared according to the traditional manufacturing method in the laboratory of the Korean Ginseng and Tobacco Research Institute (Daejeon, Korea). The white and red ginsengs were ground to pass an 80 mesh sieve and stored at −18 °C until used.

2.2. Isolation of phenolic compounds

Free phenolic, esterified, and insoluble-bound phenolic compounds were extracted and fractionated as described by Krygier et al. (1982a). Five grammes of sample powders were extracted six times in a Polytron, each with 20 ml of mixture of methanol–acetone–water (7:7:6) at room temperature. After centrifugation, the combined extracts were analyzed for free phenolic acids and soluble phenolic esters, and the residue was reserved for determination of insoluble-bound phenolic acids. The combined supernatants were concentrated to 20 ml using a rotary vacuum evaporator. Then, the aqueous suspensions was adjusted to pH 2 with 6 N HCl and centrifuged to separate a cloudy precipitate. The clear supernant was extracted five times with hexane at a hexane to water phase ratio of 1:1 to remove free fatty acids and other lipid contaminants. The free phenolic acids were extracted six times with diethyl ether–ethyl acetate (DE/EA, 1:1) at a solvent to water phase ratio of 1:1. The extracts were then dehydrated with anhydrous sodium sulphate, filtered, and evaporated to dryness under vacuum at 30 °C. The dry residues were transferred into vials containing a known amount of internal standard (*n*-tetracosane or *n*-eicosane) by using ether–ethyl acetate as the solvent and dried before silylation under nitrogen at room temperature.

The moist sodium sulphate was washed free of phenolic esters, and the washing was combined with the above water phase and the cloudy precipitate. The esters were hydrolyzed with 20 ml 4N NaOH for 4 h under nitrogen and at room temperature. Hydrolysates were acidified to pH 2, and liberated phenolic acids extracted with EE/EA as described.

The residues from the methanol–acetone extractions were hydrolyzed directly with 10 ml of 4 N NaOH under the same conditions as the esters. After acidification and centrifugation, the clear supernatants were extracted with hexane and then with diethyl ether–ethyl acetate as described earlier.

2.3. Purification of phenolic acid extracts

The solvents in extracts containing phenolic acids were distilled to dryness under nitrogen and distilled water was added to solublize the phenolic acids. The phenolic acids were purified with a C18 Sep-Pak solid phase extraction cartridge according to the method developed by Rouseff, Dettweiler, Swaine, Naim, and Zehavi (1992).

2.4. TMS derivatization of phenolic acids

To obtain trimethylsilated (TMS) derivatives of phenolic acids, the eluted portion of phenolic acids from the cartridge was dried under nitrogen. Then 0.3 ml pyridine and 0.2 ml of bis(trimethylsilyl)trifluoroacetamide (BASFTA) plus 1% trimethylchorosilane (TMCS) (Pierce, USA) were added and the mixture left for 15 min at 80 °C in a water bath.

2.5. Gas chromatography

The trimethylsilyl (TMS) derivatives of the phenolic acids were separated on a gas chromatograph (Hewlett Packard) equipped with a flame ionization detector. A DB-5 capillary column (30 m×0.25 mm, 0.20 μm, J&W Scientific, Folsom, CA) was employed. Head pressure of the carrier gas (helium) was 8 psig. Injection port was 260 °C. Oven temperature was programmed from 80 to 260 °C at 4 °C/min and held at 260 °C for 5 min. Mass-spectrometry and comparison of gas chromatographic retention times were used for identification of individual phenolic acids isolated from the gas chromatograph. Results were expressed as mg in 100 g samples (on a dry weight basis) by use of an internal standard (N-dodecosane) peak quantitative method. The quantifications of phenolic acids in ginsengs were done in duplicate.

2.6. Gas chromatograph/mass spectrometry

For identification, a gas chromatograph coupled to a mass spectrometer (Perkin Elmer) was used. Mass spectra were obtained by electron ionization at 70 eV. The analytical conditions were identical to those used for the gas chromatography.

3. Results and discussion

3.1. Identification of phenolic acids

Fig. 1 shows the free phenolic acids (A), esterified phenolic acids (B), and bound phenolic acids (C) extracted from white ginseng. Fig. 2 shows the gas chromatograms of authentic phenolic acids. The identification of each peak was first carried out by a gas chromatography–mass spectrometry (GC–MS), and then confirmed by comparison of gas chromatographic retention times with authentic samples. GC–MS data showed that the mass spectra of peaks 8 and 11 were both identical with that of authentic *trans*-ferulic acid Me_3Si derivative (Fig. 3). The gas chromatographic analysis, however, showed that the authentic *trans*-ferulic acid has the same retention time as peak 11. Based on this result, peak 8 was tentatively identified as the *cis* isomer of ferulic acid. To confirm this idea, we treated authentic *trans*-ferulic acid with 4 N NaOH for 4 h at room temperature to produce the *cis*-ferulic acid according to the method of Krygier et al. (1982b). After that, the *cis*-ferulic acid produced was converted to its Me_3Si derivative. Gas chromatographic analysis (data not shown) showed that the Me_3Si derivative of *cis*-ferulic acid had exactly same retention time as peak 8 in Fig. 1. Based on these results, peaks 8 and 11 were confirmed to be *cis* and *trans* isomers of ferulic acid, respectively. The mass spectra of peaks 5 and 9 were also exactly the same as that of the authentic *trans-p*-coumaric acid Me_3Si derivative. Gas chromatographic retention time of authentic *trans-p*-coumaric acid was the same as that of peak 9, indicating that peak 9 was a *trans*- isomer of *p*-coumaric acid. This result also suggested that peak 5 was most likely a *cis* isomer of *p*-coumaric acid. Alkali treatment, of *trans-p*-coumaric

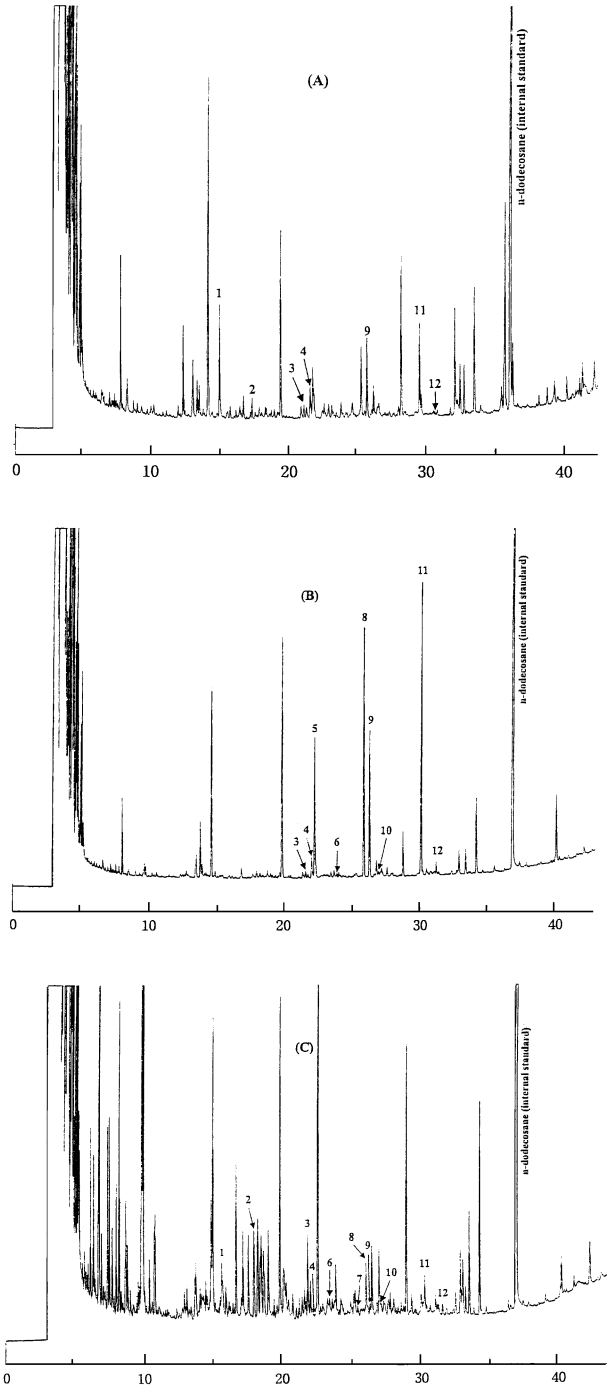

Fig. 1. Gas chromatograms of free phenolic acid Me_3Si derivatives (A), esterified phenolic acid Me_3Si derivatives (B), and insoluble-bound phenolic acid Me_3Si derivatives obtained from white Korean ginseng.

acid produced the *cis* isomer of *p*-coumaric acid. Gas chromatographic analysis, with the produced *cis*-*p*-coumaric acid Me_3Si derivative, showed that peak 5 had the same retention time as that of *cis*-*p*-coumaric acid Me_3Si derivative, confirming peak 5 as a *cis*-*p*-coumaric acid Me_3Si derivative. In this present study, 12 different phenolic acids were identified in Korean white and red ginsengs. Peaks were identified as salicylic acid (peak 1), *p*-hydroxybenzoic acid (peak 2), vanillic acid (peak 3), genistic acid (peak 4), *cis*-*p*-coumaric acid (peak 5), protocatechuic acid (peak 6), syringic acid (peak 7), *cis*-ferulic acid (peak 8), *trans*-*p*-coumaric acid (peak 9), gallic acid (peak 10), *trans*-ferulic acid (peak 11), and *trans*-caffeic acid (peak 12), respectively. Seven phenolic acids in Korean ginseng have been previously reported in the literature (Han et al., 1981; Park et al., 1994; Wee et al., 1996). The previously reported phenolic acids in Korean ginseng were vanillic acid, *trans*-*p*-coumaric, *p*-hydroxybenzoic acid, salicylic acid, genistic acids, and *trans*-ferulic acid, and caffeic acid.

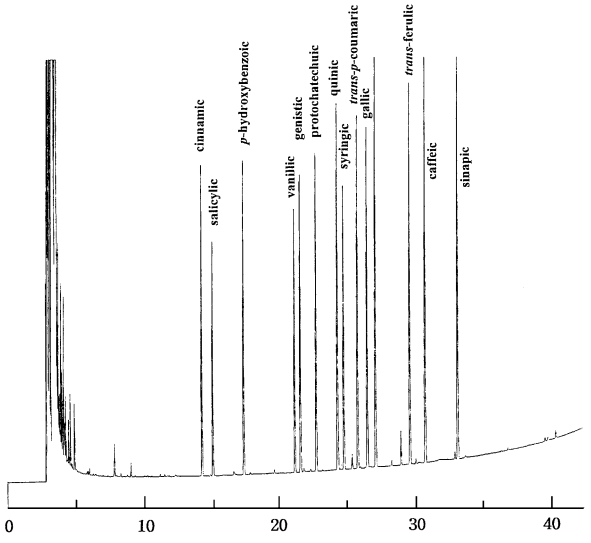

Fig. 2. Gas chromatogram of authentic phenolic acid Me_3Si derivatives.

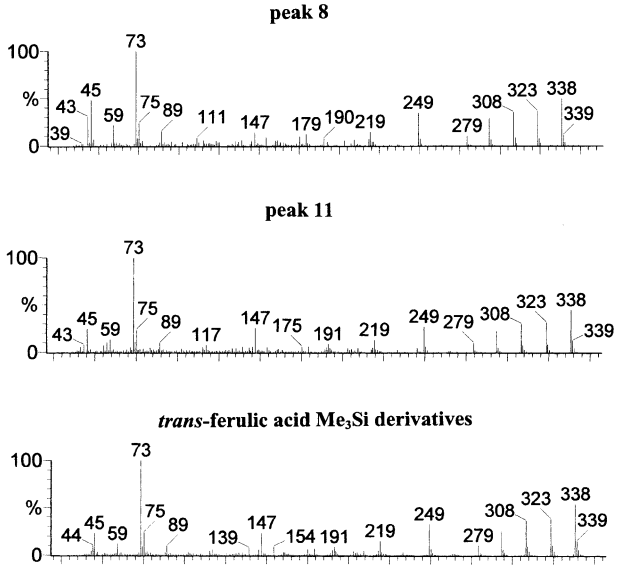

Fig. 3. Mass spectra of peak 8 and peak 11 (from gas chromatogram of Fig. 1) and authentic *trans*-ferulic acid Me_3Si derivative.

3.2. Phenolic acid contents in white and red Korean ginseng

The recovery test, during the Sep-Pak purification step, was carried out to check the accuracy of the analytical method and results are shown in Table 1. Most of the phenolic acids tested, except gallic acid and genistic acid, showed excellent recovery rates of over 86%. Gallic acid and genistic acid showed especially lower recovery rates (24.2 and 28.1% recovery, respectively). The lower recovery rates of gallic acid and genistic acid seemed to be due to their higher polarity compared with the others. This result indicated that the purification method developed by Rouseff et al. (1992) was not appropriate for gallic acid and genistic acids. Gallic acid and genistic acid, however, are reportedly not the major constituents in Korean ginseng (Park et al., 1994). Our analytical result was in good agreement with the previous report in that gallic acid and genistic acids were not present in high quantity in Korean ginseng. Thus, we believed that the analysis of the phenolic acids in white and red ginsengs did not seem to be greatly affected by the lower recovery rate of these two phenolic acids.

Tables 2 and 3 show the free, esterified, and insoluble-bound phenolic acid contents in white and red ginsengs, respectively. Total phenolic acid contents in white ginseng and red ginseng were not much different, showing 27.2 and 26.8 mg/100 g in white and red ginseng, respectively. The results showed that esterified phenolic acids were the most abundant form, followed by free, and insoluble-bound form, in decreasing order.

Total free phenolic acid contents in white and red ginsengs were 4.70 and 4.14 mg/100 g, respectively. Free *trans*-ferulic acid was the most predominant free

Table 1
Recovery of authentic phenolic acids during purification with C18 Sep-Pak solid phase extraction cartridge

Phenolic acid	Added (mg)	Recovered (mg)	Recovery (%)
Caffeic acid	0.103	0.0914	89.2
trans-*p*-Coumaric acid	0.0990	0.0947	95.7
trans-Ferulic acid	0.130	0.116	89.4
Gallic acid	0.077	0.0185	24.2
Gentisic acid	0.0970	0.0273	28.1
p-Hydroxybenzoic acid	0.0770	0.0774	101
Protochatechuic acid	0.0790	0.0794	100.5
Salicylic acid	0.0765	0.0737	96.3
Sinapic acid	0.195	0.170	86.9
Syringic acid	0.0975	0.101	103

phenolic acid, and represented 47.9 and 57.7% of total free phenolic acids in white and red ginsengs, respectively. Our present data were not in accord with the report of Park et al. (1994), who found that total phenolic acid in white ginseng was 7.47 mg/100 g and that caffeic acid was the most abundant free phenolic acid in white ginseng, reaching 2.4 mg/100 g. In our study with careful examination, caffeic acid was found at trace level in white ginseng. We could not find any other previous work reporting caffeic acid as a major constituent of free phenolic acid in Korean ginseng. The reason for the great difference in free caffeic acid content in ginsengs is unknown. However, a possible explanation for the great difference is that, since the authors analyzed phenolic acids without a column chromatographic purification step, there might be some interfering compound(s) coeluted with caffeic acid during gas chromatogtraphic analysis. This explanation is supported by the following findings. Except for caffeic acid, the contents of individual free phenolic acids in our results were remarkably similar to those of Park et al. (1994). If the caffeic acid was excluded in calculation from the report of Park et al. (1994), the total free phenolic acid content was 5.2 mg/100 g, showing great similarity to our present report of 4.7 mg/100 g. It is also interesting to note that there was a great difference in the salicylic acid content between white and red ginsengs. The salicylic acid contents in white ginseng and red ginseng were 0.94 and 0.18 mg/100 g, respectively.

Most of the phenolic acids were present in esterified form in white and red ginsengs. The esterfied phenolic acids represented 71.9 and 77.1% of total phenolic acids in white and red ginsengs, respectively. Total contents of esterified phenolic acid in white and red ginsengs were 19.6 and 20.7 mg/100 g, respectively. Ten esterified phenolic acids were identified in white and red ginsengs.

Table 2
Phenolic acids in white ginseng (mg/100 g)[a]

Peak no.	Phenolic acids	Retention time (min)	Free form	Esterified form	Insoluble-bound form
1	Salicylic	14.88	0.94±0.04	–	0.56±0.01
2	*p*-Hydroxybenzoic	17.18	0.14±0.01	–	0.50±0.05
3	Vanillic	21.00	0.11±0.04	t[b]	0.47±0.04
4	Genistic	21.45	0.22±0.04	0.32±0.04	t
5	*cis-p*-Coumaric	22.20	–	2.01±0.07	–
6	Protocatechuic	23.18	–	t	t
7	Syringic	24.50	–	–	t
8	*cis*-Ferulic acid	25.77	–	6.54±0.1	0.68±0.11
9	*trans-p*-Coumaric	26.19	1.04±0.03	2.08±0.16	t
10	Gallic	26.99	-	t	t
11	*trans*-Ferulic	30.04	2.25±0.09	8.34±0.1	0.72 ±0.04
12	*trans*-Caffeic	31.18	t	0.26±0.04	t
Total phenolic acid content			4.70±0.21	19.55±0.52	2.93±0.26

[a] Phenolic acid contents presented are average values (mg/100 g dry ginseng±S.E.) of duplicate analyses.
[b] Trace amount (less than 0.1 mg/100 g dry ginseng).

Table 3
Phenolic acid contents in red ginseng (mg/100 g)[a]

Peak no.	Phenolic acids	Retention time (min)	Free form	Esterfied form	Insoluble-bound form
1	Salicylic	14.88	0.18±0.03	t[b]	0.48±0.03
2	*p*-Hydroxybenzoic	17.18	–	–	0.26±0.03
3	Vanillic	21.00	0.18±0.03	t	0.19±0.0
4	Genistic	21.45	0.32±0.0	0.25±0.03	t
5	*cis-p*-Coumaric	22.20	–	1.52±0.01	–
6	Protocatechuic	23.18	–	t	t
7	Syringic	24.50	0.13±0.04	–	t
8	*cis*-Ferulic	25.77	–	6.97±0.03	0.45±0.04
9	*trans-p*-Coumaric	26.19	0.94±0.06	2.41±0.04	t
10	Gallic	26.99	–	–	t
11	*trans*-Ferulic	30.04	2.39±0.01	8.62±0.06	0.61±0.01
12	*trans*-Caffeic	31.18	t	0.91±0.04	t
	Total phenolic acid content		4.14±0.16	20.68±0.21	1.99±0.11

[a] Phenolic acid contents presented are average values (mg/100 g dry ginseng±S.E.) of duplicate analyses.
[b] Trace amount (less than 0.1 mg/100 g dry ginseng).

There were only 4 esterified phenolic acids (vanillic acid, *p*-coumaric acid, ferulic acid, and caffeic acid), which have been previously reported (Wee et al., 1996). In the present study, *trans*-ferulic acid, *cis*-ferulic acid, *trans*-*p*-coumaric acid, and *cis*-*p*-coumaric acid were the major esterified phenolic acids in both white and red ginseng (Tables 2 and 3). The esterified *trans*-ferulic acid contents in white ginseng and red ginsengs were 8.34, and 8.62 mg/100 g, respectively. The contents of esterified caffeic acid in white and red ginsengs were 0.26, and 0.91 mg/100 g, respectively, i.e. considerably higher in red ginseng than in white ginseng. The salicylic acid, which was found at relatively high level as free and insoluble-bound forms in both white and red ginsengs, was not found or was present at trace level, as an esterified form, in ginsengs. Even though there was a qualitative report on the esterified phenolic acid in white ginseng, the quantitative data on the esterified phenolic acids in ginsengs have never been previously reported.

Total insoluble-bound phenolic acids in white and red ginsengs were 2.93 and 1.99 mg/100 g, respectively. Five insoluble-bound phenolic acids (salicylic acid, *p*-hydroxybenzoic acid, vanillic acid, *cis*-ferulic acid, and *trans*-ferulic acid) were found at detectable level in both white and red ginsengs. Simultaneous qualitative and quantitative information on insoluble-bound phenolic acids in ginsengs has never been reported in previous publications. Ferulic acid (*cis* and *trans* isomers) was also the most predominant type in the insoluble-bound form in both white and red ginseng. The insoluble-bound *p*-hydroxybenzoic acid and vanillic acid in red ginseng were greatly lower than those in white ginseng. It is also interesting to note that *p*-coumaric acid, which was present in high concentration in free and esterified forms, was present at trace level, in insoluble-bound form, in white and red ginsengs.

In brief summary, 12 different phenolic acids as free, esterified, and insoluble-bound forms were identified in Korean ginsengs by gas chromatography–mass spectrometry and comparison of gas chromatographic retention times. The contents of individual phenolic acids in different forms in white and red Korean ginsengs were analyzed. Total phenolic acids in white and red ginsengs were 27.2 and 26.8 mg/100 g, respectively. The predominant phenolic acid in white and red ginsengs was *trans*-ferulic acid. Esterified phenolic acids represented 71.9 and 77.1% in white and red ginsengs, respectively. The identified phenolic acids are commonly found in numerous grains, plants, and foods (Krygier et al., 1982b; Labat, Morel, & Rouau, 2000; Wettasinghe, Shahidi, Amarowicz, & Abou-Zaid, 2000; Wu, Haig, Partley, Lemerle, & An, 2000; Yu, Vasanthan, & Temelli, 2001), and are therefore not unique to Korean ginsengs. However, this represents the first report of the systematic identification and quantification of phenolic acids in different forms in white and red Korean ginsengs.

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