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Analysis of Functional Constituents in Mulberry (*Morus alba* L.) Twigs by Different Cultivars, Producing Areas, and Heat Processings

Sang Won Choi[1], Yeon Jeong Jang[1], Yu Jin Lee[1], Hyun Hee Leem[2], and Eun Ok Kim[3]

[1]Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongbuk 712-702, Korea
[2]Department of Natural Product, Institute for Korea Traditional Medical Industry, Gyeongbuk 712-260, Korea
[3]Functional Food Center, Korea Institute of Science and Technology (KIST) Gangneung Institute, Gangwon 210-340, Korea

ABSTRACT: Four functional constituents, oxyresveratrol 3'-*O*-β-D-glucoside (ORTG), oxyresveratrol (ORT), *t*-resveratrol (RT), and moracin (MC) were isolated from the ethanolic extract of mulberry (*Morus alba* L.) twigs by a series of isolation procedures, including solvent fractionation, and silica-gel, ODS-A, and Sephadex LH-20 column chromatographies. Their chemical structures were identified by NMR and FABMS spectral analysis. Quantitative changes of four phytochemicals in mulberry twigs were determined by HPLC according to cultivar, producing area, and heat processing. ORTG was a major abundant compound in the mulberry twigs, and its levels ranged from 23.7 to 105.5 mg% in six different mulberry cultivars. Three other compounds were present in trace amounts (<1 mg/100 g) or were not detected. Among mulberry cultivars examined, "Yongcheon" showed the highest level of ORTG, whereas "Somok" had the least ORTG content. Levels of four phytochemicals in the mulberry twigs harvested in early September were higher than those harvested in early July. Levels of ORTG and ORT in the "Cheongil" mulberry twigs produced in the Uljin area were higher than those produced in other areas. Generally, levels of ORTG and ORT in mulberry twigs decreased with heat processing, such as steaming, and microwaving except roasting, whereas those of RT and MC did not considerably vary according to heat processing. These results suggest that the roasted mulberry twigs may be useful as potential sources of functional ingredients and foods.

Keywords: mulberry (*Morus alba*) twigs, functional constituents, cultivars, producing area, heat processing

INTRODUCTION

Mulberry (*Morus alba* L., Moraceae) has been used in traditional Chinese medicine as an anti-headache, anti-hypertensive, anti-diabetic, and diuretic agent (1). In particular, mulberry twigs have been widely used for the treatment of aching and numbness of joints in oriental medicine (2). Several prenylflavonoids, flavonoids, coumarins and stilbenes have been isolated and identified from mulberry twigs (3-6). Among them, prenylflavonoids and flavonoids have been reported as major principles for anti-obesity, antioxidant, anti-aging, and hepatoprotective activities of mulberry twigs (3-5). In addition, some coumarins and resveratrol derivatives in mulberrytwigs were found to have strong radical scavenging and anti-inflammatory activities (4,7). Thus, mulberry twigs are receiving much interest as promising sources of functional foods with health benefits.

Mulberry twigs are widely used as a promising source of well-being healthy teas, together with mulberry fruits and leaves. In addition, mulberry soups and wines made with mulberry twigs were known to have potential health benefits in folk medicine against diabetes, stroke, cough, and beriberi, etc. (1). Therefore, study on analysis of functional constituents for standardization and quality control of mulberry twig teas, soups, and wines is required.

Functional constituents in plants are affected by varieties, cultivation, maturation, storage, and processing (8-11). In particular, the content and compositions of functional constituents in different parts of mulberry trees varied considerably amongst some *Morus* species (12-14). A suitable heat processing is necessary to make the best quality processed foods using mulberry twigs for removing peculiar off-flavors like smell of greens and beans. In addition, thermal processingis known to increase functionality, palatability, and bioavailability of medicinal plants (15). To date, several studies have been

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Correspondence to Sang Won Choi, Tel: +82-53-850-3525, E-mail: swchoi@cu.ac.kr

performed on chemical analysis and screening of bioactive compounds from mulberry twigs. However, availability of information on functional constituents from mulberry twigs produced in Korea and on their quantitative changes according to varieties and processing is scarce.

The objective of this study was to isolate and identify major functional constituents from mulberry twigs, and further to quantify them by HPLC according to cultivars and processing.

MATERIALS AND METHODS

Materials and reagents

Mulberry twigs of 6 different mulberry (*Morus alba*) varieties, "Whicaso", "Yongcheon", "Guksang", "Gaeryang", "Somok", and "Cheongil", grown in the field of Sericulture and Entomology Experimental Station, Sangju, Korea, were obtained in early July and September, 2012, and shade-dried and chopped before use. Mulberry twigs produced in five different producing areas, including Yeongcheon, Sangju, Uljin, Uiseong, Gimcheon, were purchased in each regional oriental market. All solvents for HPLC analysis were obtained from Merck HPLC Grade Solvent (Merck Millipore International, Darmstadt, Germany). All other reagents used in this study were of analytical grade.

Heat processing

Dried and chopped mulberry twigs were steamed in a domestic stainless steel steamer (Kitchen-Art, Incheon, Korea) for 30 min, and roasted in an electric roaster (Dongkwang Oil Machine Co., Seoul, Korea) with constant stirring at 180°C for 5 min, and microwaved in a rotating glass container (dimensions 290 mm i.d.) at the center of a domestic microwave oven (RE-C200T, Samsung Electronics, Gyeonggi, Korea) for 5 min. Three heat pretreated mulberry twigs were dried for 12 h in a drying oven (J-300M, JISICO, Seoul, Korea) at 50±5°C and stored into plastic bags at −40°C until further analysis.

Isolation and identification of resveratrol derivatives and moracin

The dried mulberry twigs (2.0 kg) were continuously extracted with 80% aqueous ethanol (aq. EtOH) (20 L) at 40°C under an ultrasonic cleaner (Power Sonic 420, Hwashin Tech, Daegu, Korea) for 2 h, filtered and evaporated under reduced pressure. The crude EtOH extract (80.2 g) was redissolved in 80% aq. EtOH and washed with *n*-hexane. The defatted and de-pigmented EtOH extract (61.8 g) was suspended in water and successively partitioned with methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). The EtOAc fraction (9.07 g) was chromatographed on a silica

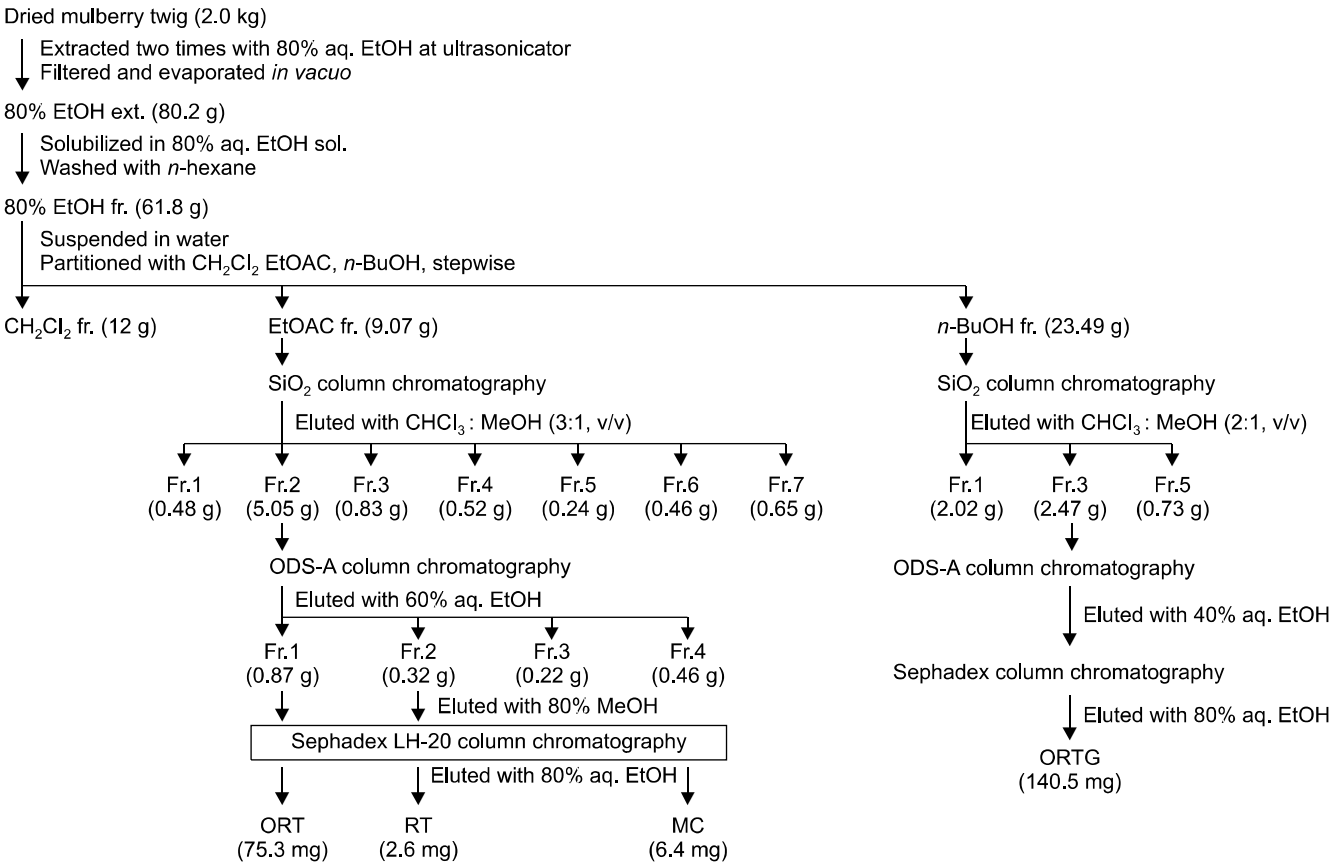

Fig. 1. Schematic procedure for isolation and purification of four functional constituents from mulberry (*Morus alba* L.) twigs.

gel (70~230 mesh, Merck Millipore International) column (6.2×40 cm) with $CHCl_3$-MeOH (3:1, v/v) as an eluent and obtained seven fractions: Fr. 1 (0.48 g), Fr. 2 (5.05 g), Fr. 3 (0.83 g), Fr. 4 (0.52 g), Fr. 5 (0.24 g), Fr. 6 (0.46 g), and Fr. 7 (0.65 g). Fr. 2 was chromatographed on an ODS-A (YMC Inc., Milford, MA, USA) column (4.0×45 cm) with 60% aq. EtOH and obtained four fractions: Fr. 1 (0.87 g), Fr. 2 (0.32 g), Fr. 3 (0.22 g), and Fr. 4 (0.46 g). Fr. 1 and 2 were finally chromatograhed on a Sephadex LH-20 column (2.4×80 cm; Pharmacia Biotech., Uppsala, Sweden) with 80% aq. EtOH and obtained Comp. 1 (ORT, 75.3 mg) from fr. 1 and Comp. 2 (RT, 2.6 mg) from fr. 2. The above fr. 4 was also chromatograhed on a Sephadex LH-20 to isolate Comp. 3 (MC, 6.4 mg). Meanwhile, *n*-BuOH fr. (23.49 g) was chromatographed on a silica gel column (6.2×40 cm) with $CHCl_3$-MeOH (2:1, v/v) and obtained five fractions; Fr. 1 (2.02 g), Fr. 2 (2.39 g), Fr. 3 (2.47 g), Fr. 4 (1.82 g), and Fr. 5 (0.73 g). Fr. 3 was also subjected to the same purification procedure on ODS-A (eluted with 40% aq. EtOH) and Sephadex LH-20 (eluted with 80% aq. EtOH) columns, and thereby isolating pure Comp. 4 (ORTG, 140.5 mg). The schematic procedure for isolation and purification of four functional constituents from mulberry twigs is shown in Fig. 1.

Identification of functional constituents

UV absorption spectra of isolated four phytochemicals (in MeOH) were obtained with a photodiode array UV-vis spectrophotometer (S-1100, Sinco, Seoul, Korea). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of compounds were measured in CD_3OD on a spectrometer (Unity Plus 500, Varian, Palo Alto, CA, USA), and chemical shifts are given as δ value with tetramethylsilane (TMS) as an internal standard. Fast-atom bombardment mass spectrometry (FAB-MS) was performed on a JMS-700 mass spectrometer: ion source, Xe atom beam; accelerating voltage, 10 kV (JEOL Ltd., Tokyo, Japan) using *m*-butyl alcohol as a mounting matrix.

Quantification of functional constituents by HPLC

Dried mulberry twigs (10 g) were extracted twice with 200 mL of 80% aq. EtOH in an ultrasonic cleaner (Bransonic 5210R-DTH, Branson, Danbury, CT, USA) for 1 h, filtered and evaporated under reduced pressure. The EtOH extract was further redissolved in 10 mL of 80% aq. EtOH and left to stand overnight at room temperature. The upper layer was taken and filled up 100 mL with the same solvent. The aliquot was properly diluted, passed through 0.45 μm membrane filter (Whatman, Maidstone, UK) and finally injected into an analytical

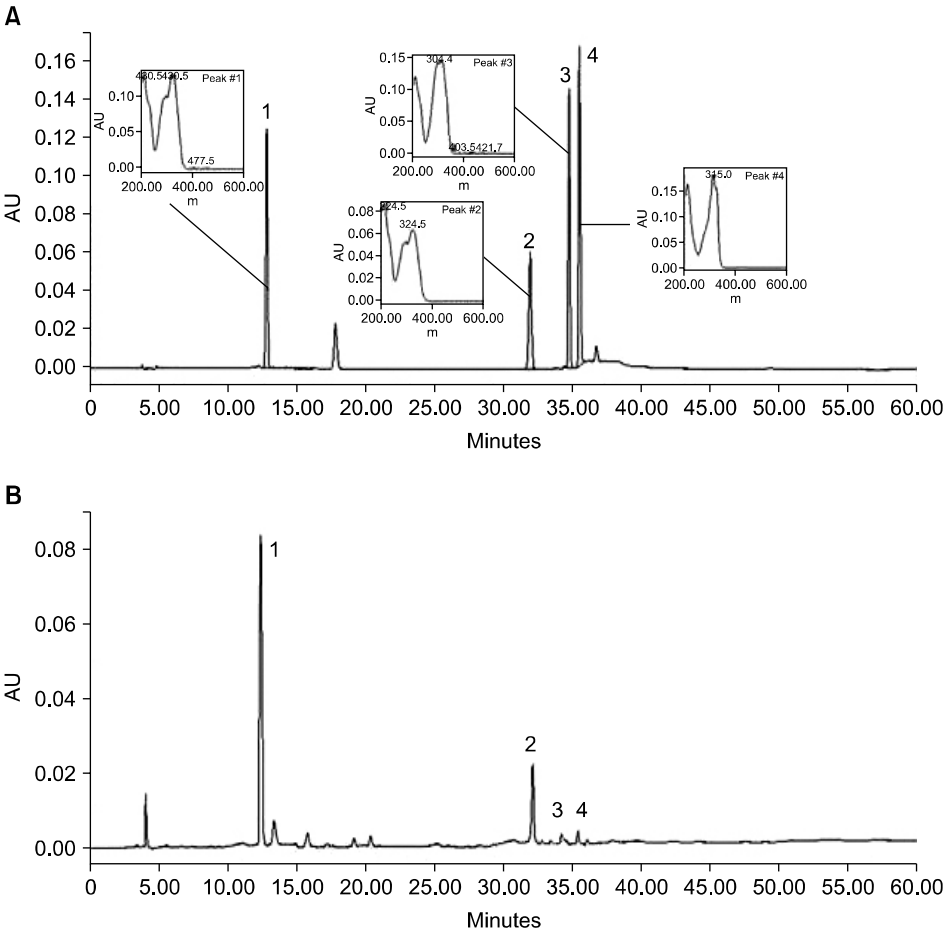

Fig. 2. HPLC chromatograms of standard resveratrol derivatives and moracin (A) and the EtOH extract (B) of mulberry (*Morus alba* L.) twigs. 1, oxyresveratrol 3`-*O*-β-D-glucoside; 2, oxyresveratrol; 3, *t*-resveratrol; 4, moracin.

HPLC. HPLC was performed on a Waters e2690/5 HPLC system (Waters, Milford, MA, USA) equipped with 2998 photodiode array detector at 310 (RT & MC) and 320 (ORTG & ORT), and autosampler. HPLC analysis was carried out using a YMC-Pack Pro C_{18} column (46 mm i.d.×250 mm, YMC Inc.) with a Guard-Pak C_{18} pre-column insert. The separation was conducted using a linear gradient of two solvent systems; solvent A, 0.05% H_3PO_4 in H_2O; solvent B, CH_3CN at a flow rate of 0.8 mL/min. The gradient elution program was performed as follows: initial 5 min run of 10% B (v/v), followed by a 45 min linear gradient to 80% B, and holding for 5 min. The injection volume was 10 μL. Individual compounds were identified by a comparison of their retention times with those of the four standard compounds isolated previously. Linear correlation coefficients were superior to 0.995 for each compound. Levels of four compounds were determined by calibration curves of the four standards (ORTG, y=1.5083x+1.4372; ORT, y=4.0271x−18.948; RT, y=8.4835x−3.8663; MC, y=7.2447x+6.6583) and expressed as mg per 100 g of dried weight of mulberry twigs. Recovery rates of four compounds were above 97%. The typical HPLC chromatograms of the four standard compounds and the 80% aq. EtOH extract of mulberry twigs are shown in Fig. 2.

Statistical analysis

Data are expressed as mean±SD of two determinations. Statistical analysis is omitted for simplicity.

RESULTS AND DISCUSSION

Isolation and identification of four functional constituents from mulberry twigs

Three resveratrol derivatives, such as oxyresveratrol 3'-*O*-β-D-glucoside (ORTG), oxyresveratrol (ORT) and *t*-resveratrol (RT), and moracin (MC) (Fig. 3) were isolated from mulberry twigs by a series of isolation procedures, including solvent fractionation, and silica gel, ODS-A and Sephadex LH-20 column chromatograpies. Their chemical structures were identified by NMR and FABMS spectral analysis, and by comparison of spectral data of the published reports (16,17). Four functional compounds have already been isolated and identified from mulberry twigsand root barks (4,6,18). Especially, Oh et al. (4) and Chang et al. (6) isolated and identified ORT and RT as major principles for antioxidant and anti-tyrosinase activities of mulberry twigs. Zhang et al. (18) and Lee et al. (19) also isolated and identified ORTG and MC derivatives from mulberry root bark and fruit. However, ORTG and MC were not yet found in mulberry twigs. Thus, this is the first report on isolation and identification of ORTG derivatives and MC from mulberry twigs. The detailed NMR and FABMS spectral data of four functional constituents from mulberry twigs were given in Table 1.

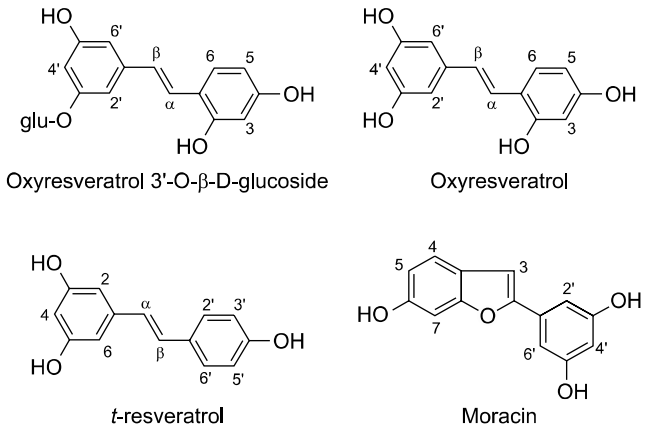

Fig. 3. Chemical structures of resveratrol derivatives and moracin isolated from mulberry (*Morus alba* L.) twigs.

Resveratrol derivatives and moracin in mulberry tree have been reported as anti-inflammatory, antidiabetic, antiaging, and antioxidant activities (6,7,18). In particular, oxyresveratrol is well-known as a tyrosinase inhibitor and has been received a potential anti-browning and skin-whitening agent in food and cosmetics, although their application to food and cosmetic industries is not implemented yet due to stability and safety (20-22). Moreover, *trans*-resveratrol, a phytoalexin produced naturally by several plants including grape and peanut when under attack by pathogens such as bacteria or fungi, has been reported to possess a variety of biological and pharmacological actions (23), and is receiving a renewed interest as a potential nutritional supplement for extending the lifespan of humans, although there is no published evidence on any clinical trial for efficacy in humans (24). Meanwhile, 2-arylbenzofuran derivatives including moracin have recently been attracted much attention as promising phytochemicals due to their anticancer, anti-inflamamtory, anti-hyperlipidemic, antimicrobial, and antioxidant activities (7,14,25,26). Thus, mulberry twigs having resveratrol derivatives and moracin are useful as promising sources of nutraceuticals and cosmeceuticals.

Quantitative changes of functional constituents in mulberry twigs by cultivars, producing area, and thermal process

Four functional constituents in mulberry twigs were quantified by HPLC according to variety, producing area, and thermal processes. As shown in Fig. 2, the three resveratrol derivatives, ORTG, ORT and RT, and MC in mulberry twigs were found in the 80% aq. EtOH extract (B) of mulberry twigs, by comparison with retention time of each standard compound (A). First, levels of the four phytochemicals in mulberry twigs from the six different

Table 1. ^{1}H- and ^{13}C-NMR spectral data of resveratrol derivatives and moracin isolated from mulberry (*Morus alba* L.) twigs

Position	ORTG	ORT	RT	MC
^{1}H-NMR				
2			6.45 (2H, d, *J*=2.5 Hz)	
3	6.77 (1H, t, *J*=2.0 Hz)	6.31 (1H, d, *J*=2.0 Hz)		6.91 (H, s)
4			6.16 (1H, t, *J*=2.5 Hz)	7.35 (H, d, *J*=8.4 Hz)
5	6.24 (1H, dd, *J*=8.4, 2.0 Hz)	6.33 (1H, dd, J=9.2, 2.0 Hz)		6.74 (H, dd, *J*=2.4 & 8.4)
6	7.43 (1H, d, *J*=2.0 Hz)	7.33 (1H, d, *J*=9.2 Hz)	6.45 (2H, d, *J*=2.5 Hz)	
7				6.90 (H, d, *J*=2.0)
2'	6.61 (2H, d, *J*=2.0 Hz)	6.45 (2H, d, *J*=2.0 Hz)	7.35 (2H, d, *J*=8.5 Hz)	6.76 (H, d, *J*=2.0)
3'			6.75 (2H, d, *J*=8.5 Hz)	
4'	6.45 (1H, t, *J*=2.0 Hz)	6.14 (1H, t, *J*=2.0 Hz)		6.25 (H, t, *J*=2.0)
5'			6.75 (2H, d, *J*=8.5 Hz)	
6'	6.61 (2H, d, *J*=2.0 Hz)	6.45 (2H, d, *J*=2.0 Hz)	7.35 (2H, d, *J*=8.5 Hz)	6.76 (H, d, *J*=2.0)
H-α	7.32 (1H, d, *J*=16.0 Hz)	7.27 (1H, d, *J*=16.4 Hz)	6.80 (1H, d, *J*=16.5 Hz)	
H-β	6.94 (1H, d, *J*=16.0 Hz)	6.82 (1H, d, *J*=16.4 Hz)	6.96 (1H, d, *J*=16.5 Hz)	
Glucose				
H-1	4.91 (1H, d, *J*=7.5 Hz)			
H-2	3.41 (1H, t, *J*=7.7 Hz)			
H-3	3.44 (1H, m, *J*=8.3 Hz)			
H-4	3.30 (1H, t, *J*=8.3 Hz)			
H-5	3.47 (1H, m, *J*=2.5 & 5.5 Hz)			
H-6a	3.90 (1H, dd, *J*=12.5 Hz)			
H-6b	3.71 (1H, dd, *J*=12.5 Hz)			
^{13}C-NMR				
1	120.39	116.64	141.48	
2	157.31	156.14	105.95	156.27
3	104.13	102.35	159.83	102.34
4	160.55	158.04	102.64	122.14
5	108.34	107.19	159.83	113.39
6	128.55	127.19	105.95	156.98
7				98.60
8				157.39
9				123.18
1'	142.10	140.99	129.57	133.95
2'	107.36	104.45	128.97	104.06
3'	159.69	158.37	116.66	160.09
4'	104.13	101.07	158.55	103.64
5'	159.67	158.37	116.66	160.09
6'	109.48	104.45	128.97	104.06
α	125.10	123.61	127.19	
β	127.76	125.30	130.60	
G-1	102.35			
G-2	74.97			
G-3	78.28			
G-4	71.47			
G-5	78.24			
G-6	62.60			
FABMS $[M+H]^{+}$	407	245	229	243

Chemical shift (δ ppm), coupling constant (*J*) expressed in Hz in parenthesis and measured in the solvent CD_3OD, taking TMS as an internal standard. FABMS spectra were determined by *m*-butyl alcohol as a matrix.
ORTG, oxyresveratrol 3'-*O*-β-D-glucoside; ORT, oxyresveratrol; RT, *t*-resveratrol; MC, moracin.

mulberry varieties harvested at early July were shown in Table 2. Among four compounds detected, ORTG was a major predominant compound in mulberry twig, and its contents ranged from 23.7 to 105.5 mg/100 g of dry weight of mulberry twigs. In contrast, other compounds were nearly found in mulberry twigs. Of six different mulberry cultivars, "Yongcheon" cultivar (105.5 mg%) had the greatest amount of ORTG in mulberry twigs, followed by "Guksang" (87.8 mg%)>"Whicaso" (84.4 mg%)>"Cheongil" (46.8 mg%)>"Gaerayng" (38.3 mg%) >"Somok" (23.7 mg%), in descending order. Moreover, the contents of four compounds in mulberry twigs generally increased by an increase of harvest time, and especially three minor compounds in the mulberry twigs harvested at early July increased at early September. Thus, there are considerable differences in phytochemical contents of mulberry twigs in relation to mulberry varieties and harvest time. Additionally, levels of four compounds of mulberry twigs produced in several different cultivated areas were presented in Table 3. Among five different mulberry twigs examined, ORTG and ORT were found as major compounds in mulberry twigs, otherwise ORTG was a major abundant compound in mulberry twigs harvested in early July. Levels of ORTG and ORT

Table 2. Contents of resveratrol derivatives and moracin of mulberry twigs harvested at early July according to mulberry (*Morus alba* L.) cultivars

Cultivar	Content (mg/100 g, dried weight)			
	ORTG	ORT	RT	MC
Whicaso	84.4±5.0 (165.6±3.5)	Tr (39.5±2.3)	Tr[1)] (1.2±0.3)	Tr (1.3±0.1)
Gaerayng	38.3±3.1 (72.4±3.6)	Tr (18.5±1.2)	Tr (1.4±0.4)	Tr (0.7±0.1)
Guksang	87.8±4.9 (162.4±3.6)	Tr (30.5±2.1)	Tr (1.3±0.3)	Tr (2.8±0.3)
Yongcheon	105.5±11.4 (198.5±5.2)	ND[2)] (49.3±2.9)	ND (1.4±0.2)	ND (3.3±0.5)
Cheongil	46.8±5.2 (93.0±3.1)	Tr (41.3±2.4)	Tr (0.9±0.1)	Tr (1.2±0.2)
Somok	23.7±2.3 (43.0±2.0)	ND (10.4±1.4)	Tr (0.8±0.1)	Tr (0.8±0.1)

All data are mean±SD of two determinations.
Statistical analysis is omitted for simplicity.
Parentheses represent content of functional constituents of mulberry twigs harvested in early September.
ORTG, oxyresveratrol 3'-*O*-β-D-glucoside; ORT, oxyresveratrol; RT, *t*-resveratrol; MC, moracin.
[1)]Trace(< 0.1 mg/100 g). [2)]Not detected.

of mulberry twigs varied greatly with producing areas of mulberry tree. However, levels of RT and MC were very small amount at below 1.5 mg%, and did not considerably vary among producing areas. It was already known that raw plants contain mostly glycoside forms of phytochemicals and low percentage of aglycone, and aglycone forms increased with growth and aging of plants (27). Thus, this fact suggest following possibility that ORTG in mulberry twigs was partly converted into its corresponding aglycone, ORT, by enzymatic conversion during drying and aging processes, as compared to mulberry twigs harvested at early July, as shown in Table 2.

Meanwhile, quantitative changes of the four functional constituents in mulberry twigs from "Cheongil" cultivar, which is widely used for production of mulberry fruit, leaf, and twig, were investigated according to three different thermal pre-treatments, such as steaming, roasting, and microwaving processes. As shown in Table 4, levels of ORTG and ORT of mulberry twigs decreased with a heat processing except a roasting process, and especially their levels greatly decreased by steaming process. In contrast, there were not big quantitative changes in levels of RT and MC in mulberry twigs in relation to thermal process. Thus, the quantitative changes of resveratrol derivatives and moracin in mulberry twigs are greatly affected by a thermal process. In particular, we found that oxyresveratrol derivatives are very susceptible to heat treatments, and their quantitative changes of mulberry twigs were dependent on heat treatments including roasting, steaming and microwaving. A previous study demonstrated that the heat treatment at a high temperature converts glycosides in plants into its

Table 3. Contents of resveratrol derivatives and moracin of "Cheongil" mulberry (*Morus alba* L.) twigs produced in several different areas

Producing area	Content (mg/100 g, dried weight)			
	ORTG	ORT	RT	MC
Yeongcheon	85.5±3.3	68.4±2.9	1.0±0.1	0.7±0.1
Sangju	174.5±4.6	88.5±3.8	0.8±0.2	1.0±0.2
Uljin	196.7±6.3	106.7±5.1	0.7±0.1	0.7±0.2
Uiseong	92.7±4.2	21.7±1.5	0.3±0.1	1.2±0.2
Gimcheon	95.6±3.5	90.7±3.1	1.1±0.3	0.9±0.3

All data are mean±SD of two determinations.
Statistical analysis is omitted for simplicity.
ORTG, oxyresveratrol 3'-*O*-β-D-glucoside; ORT, oxyresveratrol; RT, *t*-resveratrol; MC, moracin.

Table 4. Quantitative changes of resveratrol derivatives and moracin in "Cheongil" mulberry (*Morus alba* L.) twigs produced in Uljin according to heat processes

Heat processing	Content (mg/100 g, dried weight)			
	ORTG	ORT	RT	MC
Control	196.7±6.3	106.7±5.1	0.7±0.1	0.7±0.2
Steaming (30 min)	65.6±3.1	66.7±2.9	0.9±0.2	1.2±0.3
Roasting (5 min)	189.1±4.9	96.5±3.8	0.6±0.2	0.7±0.2
Microwaving (5 min)	172.5±3.3	72.6±2.8	0.6±0.1	0.8±0.2

All data are mean±SD of two determinations.
Statistical analysis is omitted for simplicity.
ORTG, oxyresveratrol 3'-*O*-β-D-glucoside; ORT, oxyresveratrol; RT, t-resveratrol; MC, moracin.

corresponding aglycones (9). However, the conversion of ORTG into ORT by a heat processing was not occurred in mulberry twigs. Thus, the conversion of glycosides of plants into aglycones varied considerably according to chemical structures of phytochemicals in plants. Additionally, aglycones of resveratrol and moracin have been reported to have higher biological activity and bioavailability than their corresponding glycosides (7,28). These results suggest that the roasted mulberry twigs with higher content of ORT without grassy and beany flavors may be useful as potential sources of functional foods.

In conclusion, the three resveratrol derivatives and moracin were first isolated and identified from mulberry twigs. Their contents and compositions were varied considerably with cultivars and processing. In particular, mulberry twigs with higher amounts of resveratrol derivatives and moracin could be useful as potential sources of functional foods. Quantitative analysis of functional constituents in mulberry twigs is necessary to set up quality control system for standardization and biological evaluation of processed foods using mulberry twigs and their extracts. Further study on preparation of high quality mulberry twigs increasing functionality, platability, and bioavailability by microbial fermentation is now under way.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Lee SI. 1981. *Bonchohak*. Haklimsa, Anyang, Gyeonggi, Korea. p 136-137.
2. Zhu YP. 1998. *Chinese materia medica: Chemistry, pharmacology and applications*. Harwood Academic Publishers, Newark, NJ, USA. p 273-274.
3. Ko HH, Yu SM, Ko FN, Teng CM, Lin CN. 1997. Bioactive constituents of *Morus australis* and *Broussonetia papyrifera*. *J Nat Prod* 60: 1008-1011.
4. Oh H, Ko EK, Jun JY, Oh MH, Park SU, Kang KH, Lee HS, Kim YC. 2002. Hepatoprotective and free radical scavenging activities of prenylflavonoids, coumarin, and stilbene from *Morus alba*. *Planta Med* 68: 932-934.
5. Hu X, Wu JW, Zhang XD, Zhao QS, Huang JM, Wang HY, Hou AJ. 2011. Isoprenylated flavonoids and adipogenesis-promoting constituents from *Morus nigra*. *J Nat Prod* 74: 816-824.
6. Chang LW, Juang LJ, Wang BS, Wang MY, Tai HM, Hung WJ, Chen YJ, Huang MH. 2011. Antioxidant and antityrosinase activity of mulberry (*Morus alba* L.) twigs and root bark. *Food Chem Toxicol* 49: 785-790.
7. Chung KO, Kim BY, Lee MH, Kim YR, Chung HY, Park JH, Moon JO. 2003. In-vitro and in-vivo anti-inflammatory effect of oxyresveratrol from *Morus alba* L. *J Pharm Pharmcol* 55: 1695-1700.
8. Lee JY, Park KS, Choi SW. 2005. Changes in flavonoid contents of safflower leaf during growth and processing. *J Food Sci Nutr* 10: 1-5.
9. Kim EO, Lee JY, Choi SW. 2006. Quantitative changes in phenolic compounds of safflower (*Carthamus tinctorius* L.) seeds during growth and processing. *J Food Sci Nutr* 11: 311-317.
10. Kim EO, Yu MH, Lee KT, Yoon KS, Choi SW. 2009. Effect of thermal pretreatment on the functional constituents of waxy corn (*Zea mays* L.). *Food Sci Biotechnol* 18: 1336-1341.
11. Lee WJ, Choi SW. 2012. Quantitative changes of polyphenolic compounds in mulberry (*Morus alba* L.) leaves in relation to varieties, harvest period, and heat processing. *Prev Nutr Food Sci* 17: 250-258.
12. Ni G, Zhang QJ, Zheng ZF, Chen RY, Yu DQ. 2009. 2-Arylbenzofuran derivatives from *Morus cathayana*. *J Nat Prod* 72: 966-968.
13. Wang L, Yang Y, Liu C, Chen RY. 2010. Three new compounds from *Morus nigra* L. *J Asian Nat Prod Res* 12: 431-437.
14. Tan YX, Gong T, Liu C, Chen RY, Yu DQ. 2010. Five new 2-arylbenzofuran derivatives from *Morus wittiorum*. *Chem Pharm Bull (Tokyo)* 58: 579-581.
15. Kwon JY, Lee KT, Yun TM, Choi SW. 2004. Effect of heat pretreatment on the functional constituents of rice germ. *J Food Sci Nutr* 9: 330-335.
16. Qiu F, Komatsu K, Kawasaki K, Saito K, Yao X, Kano Y. 1996. A novel stilbene glucoside, oxyresveratrol 3'-*O*-β-glucopyranoside, from the root bark of *Morus alba*. *Planta Med* 62: 559-561.
17. Kanchanapoom T, Suga K, Kasai R, Yamasaki K, Kamel MS, Mohamed MH. 2002. Stilbene and 2-arylbenzofuran glucosides from the rhizomes of *Schoenocaulon officinale*. *Chem Pharm Bull (Tokyo)* 50: 863-865.
18. Zhang M, Chen M, Zhang HQ, Sun S, Xia B, Wu FH. 2009. In vivo hypoglycemic effects of phenolics from the root bark of *Morus alba*. *Fitoterapia* 80: 475-477.
19. Lee YJ, Kim EO, Choi SW. 2011. Isolation and identification of antioxidant polyphenolic compounds from mulberry (*Morus alba* L.) seeds. *J Korean Soc Food Sci Nutr* 40: 517-524.
20. Zheng ZP, Tan HY, Wang M. 2012. Tyrosinase inhibition constituents from the roots of *Morus australis*. *Fitoterapia* 83: 1008-1013.
21. Kim M, Meng X, Kim A, Wang M, Simon JE. 2006. Developing a long-lasting tyrosinase inhibitor from *Morus alba* L. *Cosmet Toiletries* 121: 91-102.
22. Li H, Cheng KW, Cho CH, He Z, Wang M. 2007. Oxyresveratrol as an antibrowning agent for cloudy apple juices and fresh-cut apples. *J Agric Food Chem* 55: 2604-2610.
23. Roupe KA, Remsberg CM, Yanez JA, Davies NM. 2006. Pharmacometrics of stilbenes: seguing towards the clinic. *Curr Clin Pharmacol* 1: 81-101.
24. Marchal J, Pifferi F, Aujard F. 2013. Resveratrol in mammals: effects on aging biomarkers, age-related diseases, and life span. *Ann N Y Acad Sci* 1290: 67-73.
25. Kang J, Chen RY, Yu DQ. 2006. Five new diels-alder type adducts from the stem and root bark of *Morus mongolica*. *Planta Med* 72: 52-59.
26. Ni G, Zhang QJ, Wang YH, Chen RY, Zheng ZF, Yu DQ. 2010. Chemical constituents of the stem bark of *Morus cathayana*. *J Asian Nat Prod Res* 12: 505-515.
27. Lee JH, Doo EH, Kwon DY, Park JB. 2008. Functionalization of isoflavones with enzymes. *Food Sci Biotechnol* 17: 228-233.
28. Sjin NH, Ryu SY, Choi EJ, Kang SH, Chang IM, Min KR, Kim YS. 1998. Oxyresveratrol as the potent inhibitor on dopa oxidase activity of mushroom tyrosinase. *Biochem Biophys Res Commun* 243: 801-803.