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Far Infrared Ray Irradiation Stimulates Antioxidant Activity in *Vitis flexuosa* THUNB. Berries

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ABSTRACT : Wild grapes have been used as traditional medicinal use and alcoholic beverage production in Korea. The objective of this study is to improve antioxidant properties in Sae-muru by far infrared ray (FIR) treatment, with expecting potential benefits of FIR treatment for wild grape products during manufacturing processes. FIR treatment in berries induced increased content of catechin, epicatechin gallate, epigallocatechin gallate, gallic acid, rutin, ellagic acid, and resveratrol, while content of epicatechin and epigallocatechin was decreased. Although FIR treatment resulted either increased or decreased chemical component groups, presenting in HPLC chromatograms, antioxidant activity in Sae-muru extract was significantly increased by the FIR treatment. Our results suggest that FIR treatment should be an efficient process in the production of high content of bioactive molecules in Sae-muru.

Key words : antioxidants, catechins, far infrared ray, resveratrol, wild grape

INTRODUCTION

Wild grapes are broadly distributed in the Korean Peninsula and distinguished by differences of morphology, genotype, and/or distributional territory, named as Muru (*Vitis coignetiae* PULLIAT), Sae-muru (*Vitis flexuosa* THUNB), Wang-muru (*Vitis amurensis* RUPRECHT), Seom-muru (*Vitis coignetiae* for. Glabrescens (NAK) HARA), Gamague-muru (*Vitis ficifloia* var. sinuate (REGEL) HARA), and Chunggamagur-muru (*Vitis ficifloia* var. glabrata (NAK.) W. LEE) (Park *et al.*, 2005a; Park *et al.*, 2005b; Park *et al.*, 1993). Wild grapes have been used as a traditional medicine and alcoholic beverage production in Korea. Also, the importance of wild grapes has been constantly recognized in the aspect of genetic resources, utilizing as a parental plant for increasing functional compounds and tolerating environmental stresses such as disease and cold temperature. It has been reported that wild grapes are rich of antioxidants (Choi *et al.*, 2006). In addition, grape extracts contained many medicinal compounds, which act as medicinal factors preventing cardiovascular disease, cancer, and neuronal injury (Vauzour *et al.*, 2007). Numerous polyphenols, including resveratrol (Wang *et al.*, 2002), catechins, and ellagic acid (Talcott and Lee, 2002; Losso *et al.*, 2004), have known as functional compounds in grapes for human health. Phenolic content in plant extracts is positively correlated with antioxidant activities. The phenolic content in grapes varies in dependant upon manufacturing processes for food products (Talcott and Lee, 2002; Wang *et al.*, 2002), as well as the content is dependant upon grape species (Mullen *et al.*, 2007).

Several reports were shown that far infrared ray (FIR) irradiation induces increase of antioxidant activity (Lee *et al.*, 2003; Kim *et al.*, 2006; Lee *et al.*, 2006). FIR is known to an electromagnetic wave, ranging around 4 to 15 ㎛ of the wave length. It has known that FIR treatment during extraction of phenolics from plant cells stimulates exudation of chemical components in cells without destruct cells by radiant heat (Niwa *et al.*, 1991) and breaks covalent bonds of polymerized phenolics resulting release of activate low molecular weight natural antioxidants (Niwa *et al.*, 1988).

The objective of this study is to determine antioxidant activities in a wild grape by FIR treatment, with expecting potential benefits of FIR treatment for wild grape products during manufacturing processes. In addition, we investigated contents of catechins in the FIR treatments.

MATERIALS AND METHODS

Plant materials

Berries of Sae-muru (*Vitis flexuosa* THUNB) were collected in a mountainous area of Youngwoel, Gangwon,

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Korea in September 2006.

FIR irradiation to grape berries.

Fresh Sae-muru berries (25 g × 4) were quickly rinsed with distilled deionized water. The berry samples were irradiated FIR into a FIR radiation chamber (Korean Energy Co. Seoul, Korea), emitting 2 to 14 ㎛ wave length. During FIR irradiation, temperature was kept to 70℃ by 35 voltage set and treatment time was 30 min. The samples treated in FIR were then dried in a 70℃ oven for 5 days. As a comparison treatment control, Sae-muru berries (100 g F.W.) were dried in a 70℃ oven for 5 days.

Extraction of Sae-muru components

Prepared samples were dissolved in 2 L of 80% methanol and kept in a dark room at ambient temperature for 3 days in order to extraction. The extraction procedures were triplicates with adding the same amount (2 L) of 80% methanol. Samples were then filtered with a Whatman no. 4 filter paper and the solution was removed by using a rotary evaporator set in 40℃. The crude extracts were dissolved in 1 L of distilled deionized-water and fractionated with organic solvents, *n*-hexane, ethyl acetate, and *n*-butanol, as following the method described by Eom *et al.* (Eom *et al.*, 2006).

Total phenolic and flavonoid contents, DPPH scavenging activity, and saccharinity

Total phenolic contents of methanolic crude extracts and their subfractions were determined on the basis of gallic acid concentrations, as following a method described by Kim *et al.* (Kim *et al.*, 2007), with slight modification. Briefly, 1 mL of diluted samples was added into 9 mℓ of distilled deionized water. A reagent blank was prepared using distilled deionized water. Folin-Ciocalteu phenol (1 mL) was added to sample solution and voltexed. After 5 minute stabilization, 10 mℓ of Na_2CO_3 (7% in water) was added into the sample and voltexed. After 30 minutes incubation in ambient condition, the absorbance was measured at 725 ㎚.

Total flavonoid contents of sub-fractioned grape extracts were determined on the basis of rutin concentrations. Shortly, 1 mL (2 ㎎/ mL) of samples was mixed with 10 mL of diethylene glycol and added to 0.1 mL of 1N-NaOH solution. After shaking the mixture, the sample was incubated for 30 min at a 37℃ water bath and measured to its absorbance at 420 ㎚.

DPPH radical scavenging activity of grape samples was carried out as described by the previous method (Brand-Williams *et al.*, 1995). A series of sample concentrations (1 mL), including 125, 250, 500, 1,000, and 2,000 ㎍/mL, was added to 4 mL of 1.5×10^{-4} M of DPPH in 80% methanol. The mixed solution was shaken and incubated for 30 min at a room temperature. Absorbance was measured for DPPH remaining at 517 ㎚.

Saccharinity was determined by a master refractometer (Atago Co. Tokyo, Japan). Sample extracts was dissolved in 80% methanol (1 ㎎/mL). One drop of the samples was mounted on the instrument and saccharinity was recorded.

HPLC analysis

Sae-muru extracts were analyzed by a HPLC system (CBM-20A, Shimadzu Co. Ltd., Japan) with two gradient pump systems (LC-20AT, Shimadzu), a UV-detector (SPD-10A, Shimadzu), an auto sample injector (SIL-20A, Shimadzu) and a column oven (CTO-20A, Shimadzu). A prevailed C18 column (5 ㎛, 150 × 4.6 ㎜, Alltech. Inc. Deerfield, IL, USA) was used. The flow rate of mobile phase solution was 1.0 mL/min. The mobile phase solution was run by gradient system as following description; solution A (0.4%, v/v, formic acid in distilled deionized water) and solution B (0.4%, v/v, formic acid in acetonitrile), with a gradient elution programmed to 0 to 5% of solution B for 0 - 1 min, 5 to 10% of solution B for 1 - 45 min, 10 to 20% of solution B for 45 - 65 min. Sample (10 ㎎/mL) injection volume was 10 μL. Peaks were monitored at 280 ㎚. Standard chemicals including catechins (catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate), gallic acid, rutine, ellagic acid, and resveratrol were analyzed using the HPLC followed as above condition.

RESULTS AND DISCUSSION

Temperature control of a FIR chamber

It has been known that temperature and treatment time of FIR irradiation are important factors eluting maximized antioxidants in certain plant tissues, including Green tea (Kim *et al.*, 2006), Rice hull (Lee *et al.*, 2003), Sesame meal (Lee *et al.*, 2005) and Paprika (Park and Kim, 2007). We recorded temperatures in a FIR chamber used in this experiment by changing voltages. We found that temperature in the FIR chamber was quite stable in each voltage, with slight increase temperature when treatment time was lasted until one hour. We determined 35v, maintaining 70℃ in the chamber for Sae-muru treatment (Fig. 1). The determination of temperature was supposed by which our previous results using a red grape (*Vitis vinifera* cv. Campbell Early) in FIR treatment were shown to an efficient condition eluting maximized antioxidant activity (unpublished data).

HPLC chromatograms of Sae-muru extracts between non-FIR and FIR treatments

As compared to the berry extract of non-FIR treatment control, berry extract of FIR treatment was shown that 5 peaks on

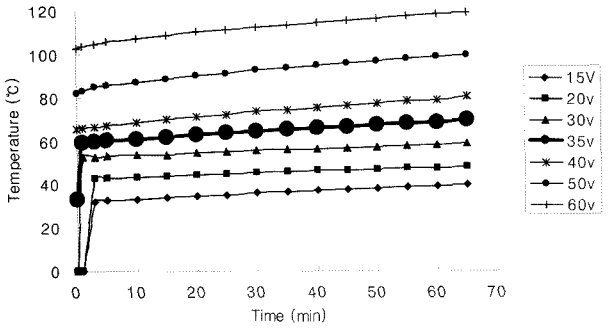

Fig. 1. Relationship between voltage and temperature of a FIR chamber used. FIR treatment condition presenting a bold line in this graph was applied to Sae-muru berries.

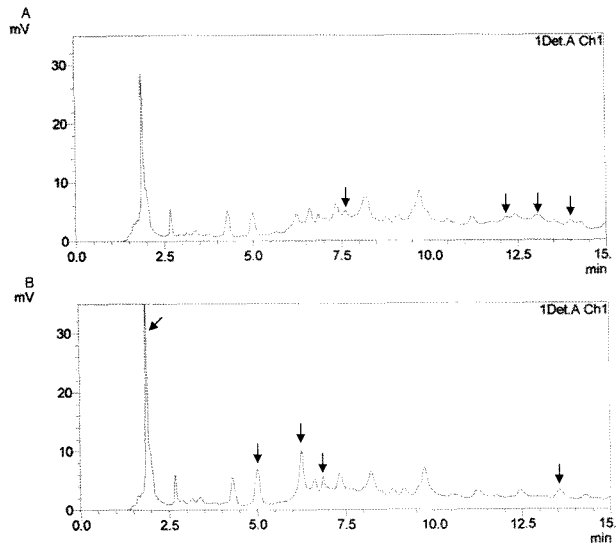

Fig. 2. HPLC chromatograms of Sae-muru crude extracts (2 ㎎/㎖). A: non-FIR treatment control. B: FIR treatment. Arrows in each chromatogram indicate increased % area in the comparison of A and B.

HPLC were significantly increased values, presenting retention time 1.915, 4.937, 6.240, 6.830, and 13.608 min (Fig. 2). On the other hand, several chemical components in non-FIR treatment control, presenting retention time 7.585, 12.179, 13.062, and 14.002 min, were decreased when FIR treatment was conducted. Based on our results, it seems obvious that FIR treatment in plant materials induces fluctuation of chemical elution, although we did not identify individual chemical components shown to the distinct content changes either increase or decrease by the effect of FIR treatment.

Content of catechins, gallic acid, rutin, ellagic acid, and resveratrol

Nine standard phenolics, including catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate,

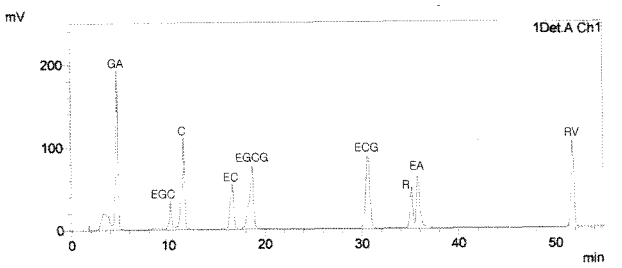

Fig. 3. Chromatogram of standard chemicals on HPLC. GA: gallic acid (500 nM). EGC: epigallocatechin (306.3 nM). C: catechin (290.3 nM). EC: epicatechin (293.3 nM). EGCG: epigallocatechin gallate (458.4 nM). ECG: epicatechin gallate (442.4 nM). R: rutin (100 nM). EA: ellagic acid (100 nM). RV: resveratrol (100 nM).

Table 1. Contents of individual phenolic compounds in methanolic extract of Sae-muru.

	Contents (㎎/g extract, n = 3)	
	Non-FIR	FIR
Gallic acid	0.933 b[1)]	3.891 a
Epigallocatechin	4.776 a	3.046 b
Catechin	1.138 a	1.379 a
Epicatechin	0.217	ND[2)]
Epigallocatechin gallate	0.121 b	0.564 a
Epicatechin gallate	0.080 b	0.165 a
Rutin	0.114 b	0.218 a
Ellagic acid	0.044 b	0.128 a
Resveratrol	0.199 b	1.002 a

[1)] The same alphabetical letters on each row were not significantly differed at Tukey's studentized analysis (LSD at p = 0.05).
[2)] ND indicates 'not detected'.

gallic acid, rutin, ellagic acid, and resveratrol, were presented on chromatogram of HPLC with successful separation (Fig. 3). Those chemicals have been commonly found in grape cultivars, especially abundant in red grape cultivars, (Talcott and Lee, 2002; Yilmaz *et al.*, 2004; Fuleki and Ricardo da Silva, 1997; Lee and Jaworski, 1990) and proved their biological activities, including anticancer, anti-cardiovascular disease, and prevention of other diseases (Steele, 2003; Briviba *et al.*, 2002).

Among 5 catechins measured in this experiment (Table 1), epigallocatechin was the most abundant compound in Sae-muru berries, containing 4.776 ㎎ in one gram extract. However, epigallocatechin was slightly decreased by FIR treatment, containing 3.046 ㎎. Epicatechin was not detected in FIR treatment, while the compound was detected 0.217 ㎎ in non-FIR treatment control, suspecting chemical degradation. Other catechins, including catechin, epicatechin gallate, and epigallo-

Table 2. FIR ray treatment effect in the contents of TPs, TFs, antioxidant activity and saccharinity in sub-fractions by organic solvents.

	Treatments	TPs (μg/mg) [1)]	TFs (μg/mg)	50% RSA (μg/mg)	Saccharinity (brix% in mg/mL)
Crude extract	Non-FIR	83.50 ± 3.18	3.82 ± 0.10	1203.78 ± 47.32	2.3
	FIR	87.95 ± 4.11	3.91 ± 0.00	978.60 ± 34.05	1.9
n-Hexane fr.	Non-FIR	92.49 ± 0.13	10.66 ± 0.10	877.75 ± 19.09	2.0
	FIR	154.70 ± 6.12	12.42 ± 0.49	460.19 ± 12.35	2.0
EtOAc fr.	Non-FIR	260.84 ± 14.56	21.82 ± 0.10	225.43 ± 11.21	1.9
	FIR	495.16 ± 10.69	42.85 ± 0.98	151.58 ± 1.97	1.8
BuOH fr.	Non-FIR	46.00 ± 0.22	2.84 ± 0.10	1881.56 ± 59.89	1.8
	FIR	100.40 ± 3.31	5.58 ± 0.10	1013.61 ± 47.72	1.8
Aqueous fr.	Non-FIR	63.75 ± 0.93	2.84 ± 0.29	1574.18 ± 40.96	1.6
	FIR	75.61 ± 2.55	3.33 ± 0.20	1195.02 ± 161.79	1.7

[1)] TPs, total phenolics; TFs, total flavonoids; RSA, radical scavenging activity; F.W., fresh weight. [2)] Values indicate average (n = 3) with standard error.

catechin gallate, were shown increased values by FIR treatment. Interestingly, the content of epigallocatechin gallate in FIR treatment was more than 4.5-fold increase compared to non-FIR treatment control.

Other chemical content, including gallic acid, rutin, ellagic acid, and resveratrol, was also shown increased concentration in FIR treatment. FIR treatment was the most efficient to extraction of resveratrol, presenting 5-fold increase (Table 1).

Antioxidant activity, phenolic content, and saccharinity

Table 2 shows comparison data between non-FIR and FIR treatments, presenting total phenolic and Flavonoid content, DPPH radical scavenging activity, and saccharinity. FIR treatment induced not only increase of bioactive molecules in the crude mathanolic extract, but also increase of them in subfractions by organic solvents from crude methanolic extract. Saccharinity in the crude extract was decreased in FIR treatment compared to non-FIR treatment. It seems clear that FIR treatment induce better extraction of bio-functional chemical components, presenting increased total phenolic and flavonoid content.

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