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Stimulating Effects of Far-infrared Ray Radiation on the Release of Antioxidative Phenolics in Grape Berries

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Abstract This research was conducted to determine the effect of far-infrared ray (FIR) irradiation of grape berries as a potential application for manufacturing grape products with a high amount of antioxidant chemicals. Two grape cultivars, the red grape cv. Campbell Early and the white grape cv. Thompson Seedless, produced increased amounts of crude extracts, in the FIR treatments compared to a non-FIR treatment control with same temperature. However, total phenolic concentrations and antioxidant activity in a 'Campbell Early' increased in the extracts following FIR treatment, whereas those of 'Thompson Seedless' did not increase significantly. High performance liquid chromatography (HPLC) analysis indicated that functional components affecting antioxidant activity were significantly increased in the extract of 'Campbell Early' following FIR treatment. Our results indicate that application of FIR treatment in heat process of grapes increases levels of antioxidative phenolic chemicals and it may help to enhance the availability of antioxidative compounds in various grape food products.

Keywords: antioxidant, 'Campbell Early', catechin, far-infrared ray, grape berriy, polyphenol, 'Thompson Seedless'

Introduction

Grapes are manufactured into wine, juice, raisins, and the fruit itself. Grape berries contain numerous functional components preventing cardiovascular disease, cancer, and neuronal injury (1). The polyphenol content of plant extracts is positively correlated with antioxidant activities, including diphenylpicryl-hydrazyl (DPPH) free radical scavenging, superoxide radical scavenging activity, and lipid peroxidation (2). However, contents of grape polyphenols are dependent upon the manufacturing process for food products (3,4) and on the grape cultivar (5). Foods contained to a higher content of polyphenols and better stabilization of the components may improve the quality of the products. An increase in functional components may be beneficial in food products. Various extraction processes from food materials may increase these components, such as controlling temperature and time, and using alternative methods such as fermentation or hydrolysis.

To increase the content of functional components in food products, several reports have shown the potential to increase extracts of antioxidants using far-infrared ray (FIR) treatments (6-9). FIR irradiation involves electromagnetic waves with wavelengths ranging from 4 to 15 μm. Effects of FIR treatment have been demonstrated in several areas, such as therapy to animals (10), growth inhibition of microorganisms in food products (11), increase of antioxidant activity exuded from plant cells (6,8,9), and stimulation of plant growth (12). It has been hypothesized that FIR treatment during extraction of polyphenols from plant cells stimulates exudation of chemical components in cells without destroying the cells by radiant heat, and breaks covalent bonds of polymerized polyphenols resulting in release of active, natural antioxidants with low molecular weight (13).

The objective of this study was to determine the content of chemical components in grapes following FIR irradiation treatment, with a focus on the potential benefits of FIR treatment for functional food products during manufacturing processes. In addition, we investigated whether certain chemical groups, depending on polarity, are mainly affected by FIR. For this research, a red grape and a white grape cultivar were used. 'Campbell Early', a red table grape, is the most popular grape cultivated in northeast Asia that is manufactured into grape juice. 'Thompson Seedless', a white grape, is also a popular grape cultivar around the world.

Materials and Methods

Materials Grapes berries, *Vitis vinifera* L. cv. Campbell Early produced in Korea and *V. vinifera* L. cv. Thompson Seedless produced in the USA, were purchased from a Korean market in Chuncheon, Korea. Gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, epicatechin gallate, and resveratrol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ellagic acid and quercetin were purchased from Wako (Osaka, Japan). Rutin was purchased from Acros (Geel, Belgium).

FIR irradiation of grape berries FIR was applied to grape berries (100 g fresh weight) in an FIR radiation chamber (Korean Energy Co., Seoul, Korea) emitting wavelengths of 3 to 1,000 μm. During FIR irradiation, temperature was held constant at 70ºC, emitting 8.4 μm wavelengths. Our preliminary experiment showed that optimal conditions of FIR treatment were 70ºC for 3 to 60 min within the range of 50 to 120ºC, based on total phenolic content (data not

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Table 1. Effect of far infrared radiation (FIR) for 30 min at 70°C between red and white grapes on total extracts (TE), total phenolics (TP), and antioxidant activities

	'Campbell Early'		'Thompson Seedless'	
	Non-FIR [1)]	FIR	Non-FIR	FIR
TE (g/100 g f.w.)	18.3	24.2	14.6	20.9
TP (μg/mg)	11.1±0.2b[2)]	33.4±1.2a	4.1±0.5a	4.8±0.4a
50% RSA[3)] (mg/mL)	5.4±0.1a	2.1±0.1b	23.0±2.6a	18.6±0.1b

[1)]Grape berries were dried in a 70°C dry oven without FIR treatment.
[2)]Different alphabetical indications on the row of each grape were significantly differed at Tukey's studentized analysis (LSD at $p<0.05$).
[3)]Radical scavenging activity; f.w., fresh weight.

shown). The samples treated in FIR were then moved into a 70°C oven for 5 days for drying. For comparison, grape berries (100 g f.w.) were dried under these same conditions, but without FIR treatment.

Extraction of grape berries Grape samples in either the FIR treatment or the non-FIR treatment as control were dissolved in 2 L of 80% methanol and kept in a dark room at ambient temperature for 3 days in order to extract antioxidative phenolics. Extraction procedures were conducted in triplicate using fresh 80% methanol. The collected sample solutions were filtered with Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, England) and the solution was removed with a vacuum rotary evaporator (Eyela Co., Tokyo, Japan). The crude extracts were dissolved in 1 L of distilled deionized water (DDW) and fractionated with the organic solvents such as *n*-hexane, ethyl acetate, and *n*-butanol following the method of Eom *et al.* (14).

Total phenolic and flavonoid content and DPPH scavenging activity Total phenolic contents of grape crude extracts and their subfractions were determined by a Folin-Ciocalteu assay using gallic acid as a standard, following Kim *et al.* (2).

The total flavonoid content of sub-fractioned grape extracts was determined using rutin as a standard (15). One mL of sample extracts (2 mg/mL) was mixed with 10 mL of diethylene glycol and added to 1 mL of 1 N NaOH solution. After shaking the mixture, the sample solution was incubated for 30 min in a 37°C water bath and measured for absorbance at 420 nm.

DPPH radical scavenging activity of grape samples was assessed as described previously (16). A series of sample concentrations (1 mL), including 125, 250, 500, 1,000, and 2,000 μg/mL, was added to 4 mL of 1.5×10^{-4} M of DPPH in methanol. The mixed solution was shaken and incubated for 30 min at room temperature. Absorbance at 520 nm was measured for the DPPH remaining.

High performance liquid chromatography (HPLC) analysis of sub-fractions of 'Campbell Early' extracts To compare chemical profiles between non-FIR and FIR treatments, the sub-fractions of 'Campbell Early' extract were analyzed by an HPLC system (CBM-20A; Shimadzu Co., Ltd., Kyoto, Japan) with 2 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 Prevail C18 column (5 μm, 150×4.6 mm, Alltech. Inc., Deerfield, IL, USA) was used. Flow rate of the mobile phase solution was 1.0 mL/min. The mobile phase solution methods followed Jo *et al.* (17) with slight modification. Solution A (0.4%, v/v, formic acid in DDW) and solution B (0.4%, v/v, formic acid in acetonitrile) were used 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, and 10 to 20% of solution B for 45-65 min. Sample (1 mg/mL) injection volume was 30 μL. Peaks were monitored at 280 nm.

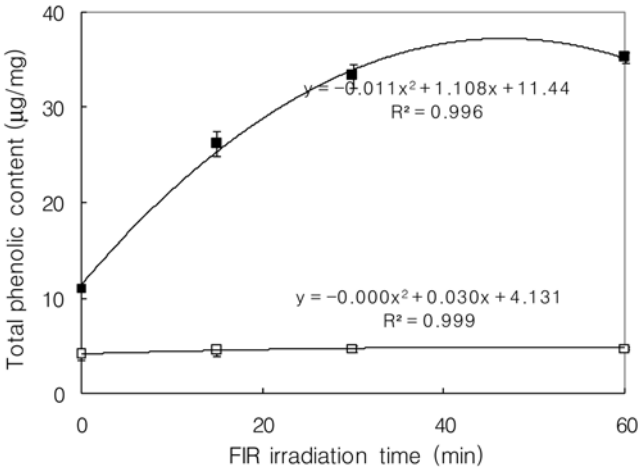

Fig. 1. Effect of FIR irradiation time on extraction of total phenolics. Total phenolics of 'Cambell Early' extract (■). Total phenolics of 'Thompson Seedless' extract (□). The error bars represent of averages.

Statistical analysis Means of statistically analyzed data were subjected to standard analysis of variance (ANOVA) procedures in SAS software (SAS version 8.02, SAS Institute, Cary, NC, USA). Significant differences among data were determined at the 5% level based on Fisher's least significant difference (LSD) tests.

Results and Discussion

Effect of FIR time treatments on total phenolics of 'Campbell Early' and 'Thompson Seedless' The content of total phenolics is higher in red grape cultivars than in their white counterparts (18). The content of total phenolics was 2.7-fold greater (11.1 vs. 4.1 μg/mg) in intact 'Campbell Early' than in intact 'Thompson Seedless' (Table 1). The effect of FIR irradiating time on the content of total

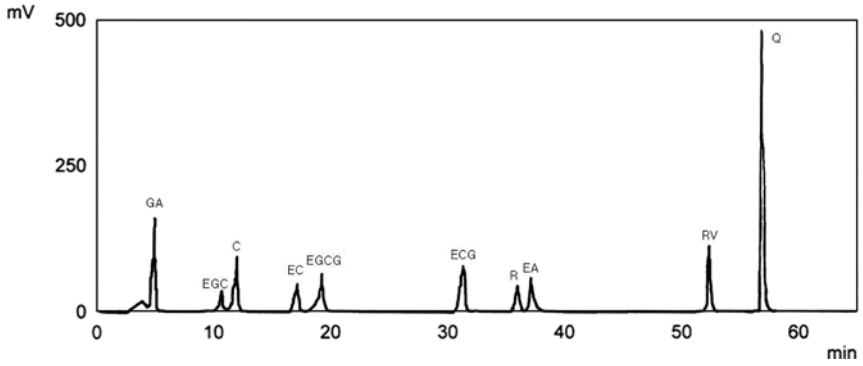

Fig. 2. HPLC chromatogram of 10 selected standard phenolics. GA, gallic acid; EGC, epigallocatechin; C, catechin; EC, epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; R, rutin; EA, ellagic acid; RV, resveratrol; and Q, quercetin.

phenolics in these 2 grapes is shown in Fig. 1. The content of total phenolics in 'Campbell Early' increased when time of FIR treatment was increased to 60 min. The content of total phenolics in 'Campbell Early' increased 3.0-fold (33.4 μg/mg) under the 30-min FIR treatment compared to the non-FIR treatment control (11.1 μg/mg). However, the increase was not significantly different between 30- and 60-min FIR treatments. For this reason, we concluded that the most efficient time for FIR in 'Campbell Early' is 30 min. In the case of 'Thompson Seedless', total phenolics did not increase significantly when the length of the FIR treatment was increased.

Effect of FIR on total exudation of grape extracts and antioxidant activity The amount of extract obtained from plant materials depends on the chemical composition of the plants, processing procedures, climate, and extracting solvents. Among the solvents for the extraction of polyphenols from plant materials, we used 80% methanol, one of the most frequently used solvents for plant extracts, to determine the effect of FIR treatment on extraction of grape berry components. As shown in Table 1, the amount of crude extract after FIR treatment was significantly increased in both 'Campbell Early' and 'Thompson Seedless' compared to the non-FIR treatment control. In the case of 'Campbell Early', the irradiated extract (24.2 g/100 g f.w.) contained higher total phenolic content (33.4 μg/mg), and showed stronger DPPH radical scavenging activity, compared to the non-FIR treatment control exhibiting 18.3 g of crude extract and 11.1 μg of total phenolics. Otherwise, in the case of 'Thompson Seedless', the amounts of crude extract (20.9 g) was increased in FIR treatment (20.9 g) compared to that of non-FIR treatment (14.6 g) and total phenolics (4.8 g) after FIR treatment was not significantly different from that of the non-FIR treatment control. The DPPH radical scavenging activity following FIR treatment in 'Thompson Seedless' increased significantly in relation to the control, yet it was still lower in comparison to the difference exhibited by 'Campbell Early'.

Chemical profiles of grape extracts on HPLC Ten standard phenolics (gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, epicatechin gallate, quercetin, rutin, ellagic acid, and resveratrol) were successfully separated on the HPLC chromatogram (Fig. 2).

Table 2. Contents of individual phenolic compounds in methanolic extracts of a red grape, 'Campbell Early'

	Contents of 10 phenolics (mg/100 g f.w. ± SE)	
	Non-FIR	FIR
Catechin	8.7±0.3b[1)]	32.2±1.4a
Epicatechin	0.9±0.2b	3.1±0.0a
Epigallocatechin	12.3±0.3b	28.3±3.2a
Epigallocatechin gallate	6.9±0.1b	21.6±2.0a
Epicatechin gallate	ND	ND
Gallic acid	6.4±2.0b	22.1±0.4a
Rutin	ND	ND
Ellagic acid	1.1±0.0	ND
Resveratrol	1.3±0.1b	7.0±0.2a
Quercetin	3.4±0.1b	5.9±0.5a

[1)]Different alphabetical letters on each row were significantly differed at Tukey's studentized analysis (LSD at $p<0.05$); ND, not detected.

Catechins have shown bioactivity in protecting against cancer, cardiovascular disease, and other diseases, as well as antioxidant activity (19). These compounds are also present in grape products, such as red wine (20), seeds and skins (21), and in dependence of grape cultivars themselves (22,23). Kim *et al.* (8) reported that the content of catechins, including epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, was increased by FIR irradiation during the manufacturing process in green tea. In the case of 'Campbell Early', our data indicated that FIR treatment increased various extraction amounts of total phenolics; certain components, including catechin, epicatechin, epigallocatechin gallate, gallic acid, and resveratrol, increased to much higher extraction levels, whereas other components, including epigallocatechin and quercetin, increased only slightly.

Contents of catechins, including catechin, epigallocatechin, epicatechin, and epigallocatechin gallate, were significantly increased by the FIR treatment (Table 2). Epicatechin gallate was not detected in 'Campbell Early' extracts. Other total phenolics measured in this experiment increased following FIR treatment, compared to the non-FIR treatment control; these included gallic acid (from 6.4 to

Table 3. Chemical composition of sub-fractioned 'Campbell Early'[1)]

	n-Hexane fr.		EtOAc fr.		*n*-BuOH fr.		Aqueous fr.	
	Non-FIR[2)]	FIR	Non-FIR	FIR	Non-FIR	FIR	Non-FIR	FIR
TE (g/100 g f.w.)	0.7	0.8	0.3	0.4	5.2	6.1	11.8	16.8
TPs (μg/mg)	12.7±1.0[3)]	57.4±1.4	101.9±2.8	158.3±4.8	12.0±0.5	33.1±1.5	2.0±0.3	8.3±1.4
TFs (μg/mg)	9.5±0.4	12.3±1.5	20.5±1.3	27.9±1.5	10.6±0.6	13.4±0.9	9.4±0.4	9.5±0.8
IC_{50} of RSA (mg/mL)	2.93±0.07	1.09±0.00	1.09±0.00	1.08±0.00	3.48±0.05	1.49±0.01	11.71±0.35	5.18±0.03

[1)]TE, total extract; TPs, total phenolics; TF, total flavonoids; RSA, radical scavenging activity; f.w., fresh weight.
[2)]Grape berries were dried in a 70°C dry oven without FIR treatment.
[3)]Mean±SE (n=3).

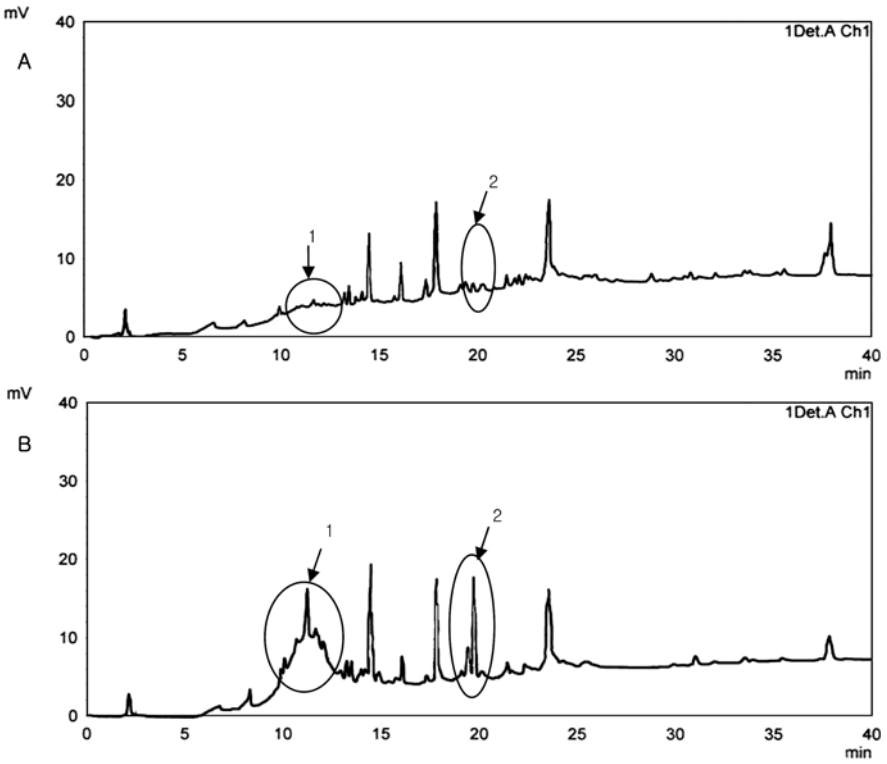

Fig. 3. HPLC profiles of sub-fractions by *n*-hexane from the extracts of non-far infrared ray treatment (A) and far infrared ray treatment (B).

22.1 mg), resveratrol (from 1.3 to 7.0 mg), and quercetin (from 3.4 to 5.9 mg). Ellagic acid was not detected in the FIR treatment, but it was detected in the control. Our results indicate that the extraction of total phenolics by FIR treatment may depend on characteristics of the chemical components.

Chemical properties of 'Campbell Early' by sub-fractionation Methanolic crude extracts contain many complex components, including both functional bioactive and non-bioactive molecules (14). The complex components may limit evaluation of bioactivity of certain molecules in food extracts. Sub-fractionation using organic solvents in methanolic crude extract of 'Campbell Early' was accomplished in order to classify antioxidant groups depending on polarity. We found that all contents of the sub-fractions increased following FIR treatment (Table 2). Among these fractions, the aqueous fraction representing most polar components had the largest difference in content between the FIR treatment (16.8 g) and the non-FIR treatment control (11.8 g). This difference may be ascribed mainly to water-soluble sugars. Contents of *n*-hexane and ethyl acetate fractions did not increase substantially. The ethyl acetate fraction had the highest content of total phenolics and antioxidant activity, although the total content was the lowest. Interestingly, although the content difference in the *n*-hexane fraction between the FIR treatment (0.8 g) and the non-FIR treatment control (0.7 g) did not increase greatly, the content of total phenolics from 12.7 to 57.4 μg and antioxidant activity from 2.93 to 1.09 mg/mL in IC_{50} increased in FIR treatment (Table 3). The reasons that non-polar components and antioxidant activity were increased by FIR treatments may be explained that heat energy by FIR breaks down either poly chained phenolics (13) or glucose groups composed in phenolic aglycons. Total flavonoid content of the sub-fractions also increased slightly following FIR treatment (Table 3). However, the content did not differ greatly,

unlike the content of total phenolics. The results may be explained that flavonoids existed simple forms in grape fruits which are less affected by FIR heat energy. Otherwise, polyphenols form poly chained structure and easily break down the structures to simple phenolic forms. As shown in Table 3, antioxidant activity in the *n*-hexane fraction of 'Campbell Early' had the largest increase by FIR treatment. We found that group 1 (9-13 min as retention times) and group 2 (19-20 min as retention times) on an HPLC chromatogram in the *n*-hexane fraction of 'Campbell Early' increased distinctly following FIR treatment, whereas the non-FIR control groups only had trace amounts (Fig. 3). Our observation suggests that natural products with low molecular weight, like epicatechin and catechin, may originate from polymeric phenols through breakage of covalent bonds of polymerized polyphenols by FIR treatment, as described by Niwa *et al.* (13). In the case of other fractions, including ethyl acetate, *n*-butanol, and water, certain peaks on HPLC chromatograms of the FIR treatment were also shown to represent increased levels in comparison analysis with standard chemicals (Fig. 2). Nonetheless, a difference in certain distinct peaks such as the *n*-hexane fraction was not observed in other fractions (data not shown).

In conclusion, it is clear that FIR treatment increased antioxidant activity by producing more total phenolics in 'Campbell Early', which contained substantial amounts of biologically active total phenolics and polymers of the total phenolics that are potentially broken into simple molecules. The effect of FIR was more efficient in red grape cultivars containing abundant total phenolics than in white grape cultivars containing smaller amounts of total phenolics. Recently, Park and Kim (24) reported that FIR treatment did not increase antioxidant activity during the drying process of paprika (*Capsicum annuum* L.), which contains smaller amounts of polymeric phenolics compared to red grapes and green tea. Therefore, the plant materials containing rich of polymeric chained phenolics will be better applicable to FIR dry process for manufacturing functionally improved food materials, such as red grapes and green tea.

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