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Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts

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Abstract

This study was carried out to evaluate the effect of heating and physical conditions of grape seeds on the antioxidant activity of their extracts. Two forms of grape seeds, whole and powdered forms, were heated at four different temperatures −50, 100, 150 and 200 °C. After heating, grape seeds were extracted with 70% ethanol (0.1 g grape seed/10 mL of 70% ethanol), and total phenol contents (TPC), radical scavenging activity (RSA) and reducing power of the extracts were determined. Thermal treatment of grape seed increased the antioxidant activity of extracts. The maximum TPC and RSA of whole grape seed extract (WGSE) were achieved when the seeds were heat-treated at 150 °C for 40 min, while that of powdered grape seed extract (PGSE) were at 100 °C for 10 min, and were greater than that of the non-treated control. Also, the reducing powers of WGSE and PGSE slightly increased at the conditions. According to the GC-MS analysis, several low-molecular-weight phenolic compounds such as azelaic acid, 3,4-dihydroxy benzoic acid, and *o*-cinnamic acid were newly formed in the WGSE heated at 150 °C for 40 min. There were slight differences in the kinds of phenolic compounds between non-heated and heated GSE. In HPLC analysis, the contents of gallocatechin gallate and caffeine in GSE significantly increased by heat treatment. These results indicated that antioxidant activity of GSE was affected by heating conditions (temperature and time) and physical conditions of grape seeds at the time of heat treatments.

Keywords: Grape seed extracts; Heating condition; Antioxidant activity; Phenolic compounds

1. Introduction

The importance of reactive oxygen and free radicals in cellular injury and the aging process has attracted increasing attention over the past 20 years (Lee, Koo, & Min, 2004). In addition, these molecules are considered to induce lipid peroxidation causing the deterioration of foods (Duthie, 1993). Reactive oxygen species in the forms of superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) are generated by normal metabolic processes or from exogenous factors and agents. Antioxidant defenses in organisms against reactive oxygen species (prooxidants and free radicals) produced during normal cell aerobic respiration may be of endogenous (enzymatic and monenzymatic) or dietary origin (vitamins, carotenoids, flavonoids, etc.) (Harman, 1995). Therefore, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytolune and tertiary butylhydroquinone have been used in food industry as antioxidants. However, the use of these synthetic antioxidants are restricted in some countries or states because of their toxic (Buxiang & Fukuhara,

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1997; Hirose et al., 1998) or carcinogenic effects (Namiki, 1990; Pokorny, 1991).

Recently, the interest of finding natural antioxidants, especially those of plant origin, has increased greatly (Jayaprakasha, Negi, Sikder, Rao, & Sakariah, 2000). Natural antioxidants derived from plants, especially phenolics such as quercetin, carnosol, thymol, catechin, and morin, etc., are of considerable interest as dietary supplements or food preservatives (Halliwell, Aeschbach, Löliger, & Aruoma, 1995). In most cases, phenolics mediate their anticarcinogenic effects by inhibiting all stages of chemical carcinogenesis, initiation, promotion and progression as well as formation of carcinogens from dietary precursors (Jang et al., 1997; Weisburger, Nagao, Wakabyashi, & Oguri, 1994).

Grape (*Vitis vinifera*) is one of the world's largest fruit crops and grape seed is a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins, and 7% complex phenols including tannins, in addition to sugars, mineral salts, etc. Proanthocyanidins of grape seed are a group of polyphenolic bioflavonoids, which are known to possess broad pharmacological activities and therapeutic potentials (Bagchi et al., 2002). Proanthocyanidins, the major polyphenols found in red wine and grape seeds, have been reported to show cardioprotective effects against ischemic reperfusion injury (Sato, Maulik, Ray, Bagchi, & Das, 1999). In addition, grape seeds are rich sources of monomeric phenolic compounds, such as (+)-catechins, (−)-epicatechin, (−)-epicatechin-3-*o*-gallate, and dimeric, trimeric and tetrameric procyanidins, which have antimutagenic and antiviral effects (Saito, Hosoyama, Ariga, Kataoka, & Yamaji, 1998). Recognition of such health benefits of catechins and procyanidins has facilitated the use of grape seed extract as a dietary supplement. The objective of this research was to elucidate the relationship between heating and physical conditions of grape seeds on the antioxidant activity of grape seed extract (GSE).

2. Materials and methods

2.1. Materials

Grape (*V. vinifera*, Campbell early) seeds were purchased from Taepyungyang Food Company in *Daegu*, South Korea, which was kept frozen at −70 °C until used. Tannic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol (HPLC grade) and 85% orthophosphoric acid (analytical grade) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Eight catechin standards - (−)-gallocatechin [(−)-GC], (+)-catechin [(+)-C], (−)-epicatechin [(−)-EC], (−)-epigallocatechin [(−)-EGC], (−)-epigallocatechin gallate [(−)-EGCG], (−)-gallocatechin gallate [(−)-GCG], (−)-epicatechin gallate [(−)-ECG] and (−)-catechin gallate [(−)-CG] - and gallic acid [G] and caffeine were purchased from Sigma Chemical Co. Folin–Ciocalteu reagent was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were GC or HPLC grade and used without further purification. ater used in HPLC and sampling was prepared with a Super Purity Water System (Purite Ltd., England) with a resistance over 17.5 MΩ/cm.

2.2. Heat treatment

The grape seeds were washed with excess water to remove adhering materials and sun-dried. Powder form of grape seeds were homogenized with a Mixer (MC - 811C, Novita, Korea) at high speed. Two forms of grape seeds (2.0 g) – whole and powder forms – were placed single layer in a Pyrex Petri dish (8.0 cm diameter) and roasted at 50, 100, 150 or 200 °C for 10, 20, 30, 40, 60, 90 or 120 min, respectively (43 treatments including control), in an electric muffle furnace (Model DMF-802, Daeil Engineering, Korea).

2.3. Preparation of 70% ethanol extracts of grape seeds

Raw or heat treated grape seeds (0.1 g) were extracted overnight with 10 mL of 70% ethanol solution in a shaking incubator (100 rpm) at room temperature. Then the extracts were centrifuged at 1000*g* for 15 min. The supernatants were filtered through a Whatman No.1 filter paper. The 70% ethanol extracts of whole (WGSE) or powder grape seeds (PGSE) were diluted 5-fold with 70% ethanol and used for the determination of antioxidant activity.

2.4. Total phenolic contents

The total phenolic contents (TPC) of GSE were determined using the method by Gutfinger (1981). The GSE (1 mL) was mixed with 0.2 mL 50% Folin–Ciocalteu reagent and 1 mL 2% Na_2CO_3, and centrifuged at 12000*g* for 5 min. The absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 750 nm after 30 min incubation at room temperature. TPC were expressed as tannic acid equivalents.

2.5. Radical scavenging activity

The effect of GSE on the DPPH radical was estimated according to the method of Lee et al. (2003). After mixing 0.9 mL of 0.041 mM DPPH in ethanol with 0.1 mL of GSE for 10 min, the absorbance was measured at 517 nm. Radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula: % DPPH radical scavenging activity = (1 − sample absorbance/control absorbance) × 100.

2.6. Reducing power

The reducing power of GSE was determined according to the method of Oyaizu (1986). GSE (1 mL), phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1.0 mL, 10 mg/mL) were mixed and incubated at 50 °C for 20 min. Trichloroacetic acid (1.0 mL, 100 mg/mL) was added to the mixture and centrifuged at 12000*g* for 5 min. The supernatant (1.0 mL) was mixed with distilled water (1.0 mL) and ferric chloride (0.1 mL, 1.0 mg/mL), and then the absorbance was measured at 700 nm.

2.7. Gas chromatography/mass spectrometry analysis of GSE

Each GSE from non-heat treated control or heat treated powder form at 110 °C for 10 min and whole form 150 °C for 40 min was dissolved in ethanol (200 mg/mL) and centrifuged at 12000*g* for 5 min to precipitate undissolved materials. The supernatant was mixed with 4 volumes of BSA [*N*,*O*-bis(trimethylsilyl)acetamide] and derivatized in a water bath (70 °C) for 15 min (Du & Ahn, 2002). The compounds in CFP extracts were identified using a gas chromatography/mass spectrometry (GC6890/MS5973, Hewlett-Packard Co., Wilmington, DE). A split inlet (100:1) was used to inject samples (5 μL) into an HP-5 column (30 m, 0.32 mm i.d., 0.25 μm film; Hewlett-Packard Co., Wilmington, DE). A ramped oven temperature was used (100 °C for 2 min, increased to 270 °C at 10 °C/min, and held for 6 min). The inlet temperature was 250 °C and the carrier gas was He at constant flow of 1.5 mL/min. The ionization potential of mass selective detector was 70 eV and the scan range was 19.1–400 *m*/*z*. Identification of compounds detected was achieved by comparing mass spectral data of samples with those of the Wiley library (Hewlett-Packard Co.).

2.8. Reverse phase-high performance liquid chromatography analysis of GSE

The levels of catechins and caffeine in the GSE were measured by HPLC (Wang, Provan, & Helliwell, 2003). The HPLC system was consisted of Shimadzu LC-6AD pumps (Shimadzu Co. Ltd., Kyoto, Japan) with a two-pump gradient system, Shimadzu SPD-10AVP UV–VIS detector, Shimadzu SIL-10ADVP auto sample injector, and Shimadzu CTO 10AVP column oven. The column was a Shim-pack VP ODS column (5 μm, 250 × 4.6 mm, Shimadzu Co. Ltd.) equipped with a Shim-pack CLC guard column (10 × 4 mm, Shimadzu Co. Ltd.). Mobile phases were consisted of 0.1% orthophosphoric acid in water (v/v) (eluent A) and 0.1% orthophosphoric acid in methanol (v/v) (eluent B). The composition of starting solvent was 80% solvent A and 20% solvent B. The solvent gradient was as follows: 0–5 min, 40% B; 5–12 min, linear gradient from 40% to 50% B; 12–27 min, 50% B; 27–30 min, linear gradient from 50% to 20% B; 30–35 min, linear gradient from 20% to 0% B. Post-run time was 5 min. Elution was performed at a solvent flow rate of 1 mL/min. Detection was accomplished with a UV–Vis detector and chromatograms were recorded at 210 nm. The column was maintained at 40 °C. The sample injection volume was 10 μL. Peaks were identified by comparing their retention times with authentic standards.

2.9. Statistical analysis

All measurements were done in triplicate, and analysis of variance was conducted by the procedure of General Linear Model using SAS software (SAS Institute, 1995). Student–Newman–Keul's multiple range tests were used to compare the differences of mean values among treatments ($P < 0.05$).

3. Results and discussion

3.1. Effects of heating conditions on the antioxidant activities of grape seeds extracts

TPC in WGSE and PGSE was significantly increased by heat treatments ($P < 0.05$) (Table 1). TPC in WGSE increased from 0.380 mM in control to 0.520 mM after heating at 100 °C for 60 min, and to 0.575 mM after 150 °C for 40 min treatment. TPC in the PGSE increased from 0.380 mM in control to 0.555 mM after 100 °C heating for 10 min, and to 0.515 mM after 50 °C for 90 min. However, heating at 200 °C decreased the TPC of WCPE and PGSE significantly. Phenolic acids are known to act as antioxidants not only because they are able to donate hydrogen or electrons but also stable radical intermediates, which prevent oxidation of various food ingredients, particularly fatty acids and oils (Cuvelier, Richard, & Berset, 1992). Grape seeds are a rich source of monomeric phenolic compounds (Saito et al., 1998), and antioxidative and radical scavenging activity of proanthocyanidins had been reported (Ariga, Koshiyama, & Fukushima, 1988; Ricardo da Silva, Darmon, Fenandez, & Mitjavila, 1991). These results indicated that phenolic compounds in GSE were liberated by heat treatments. Our previous studies (Jeong et al., 2004a; Jeong et al., 2004b) showed that simple heat treatment converted insoluble phenolic compounds to soluble phenolics but could not cleave covalently bound phenolic compounds from rice hull (Lee et al., 2003). This indicates that phenolic compounds of plants should present in different bound

Table 1
Effect of heat treatments condition and physical form on total phenolic contents (mM) of 70% ethanol extracts from whole (WGSE) and powder (PGSE) form of grape seeds extracts

Temperature	Heating time (min)								
	0	10	20	30	40	60	90	120	SEM
WGSE									
50	0.380^{bw}	0.317^{dx}	0.260^{fx}	0.303^{ey}	0.300^{ey}	0.313^{dy}	0.442^{ax}	0.330^{cx}	0.003
100	0.380^{dw}	0.326^{fx}	0.347^{cw}	0.414^{cx}	0.407^{cx}	0.520^{aw}	0.458^{bw}	0.390^{dw}	0.006
150	0.380^{dw}	0.392^{dw}	0.348^{cw}	0.444^{cw}	0.575^{aw}	0.484^{bx}	0.358^{ey}	0.319^{fy}	0.006
200	0.380^{aw}	0.254^{bz}	0.189^{cy}	0.163^{dz}	0.115^{ez}	0.179^{cz}	0.179^{ez}	0.113^{ez}	0.005
SEM	0.006	0.005	0.006	0.004	0.005	0.006	0.003	0.002	
PGSE									
50	0.380^{dw}	0.344^{ex}	0.332^{ey}	0.451^{bw}	0.424^{cx}	0.444^{bcw}	0.400^{dw}	0.515^{aw}	0.007
100	0.380^{cw}	0.555^{aw}	0.296^{dx}	0.359^{cx}	0.375^{cy}	0.378^{cy}	0.418^{bx}	0.185^{ex}	0.008
150	0.380^{cw}	0.340^{dx}	0.417^{bw}	0.427^{bw}	0.483^{aw}	0.407^{bx}	0.319^{dx}	0.196^{ex}	0.008
200	0.380^{aw}	0.190^{cz}	0.185^{cz}	0.269^{by}	0.160^{dz}	0.151^{dz}	0.067^{ey}	0.064^{ey}	0.004
SEM	0.006	0.006	0.009	0.01	0.005	0.004	0.008	0.002	

$^{a–f}$ Different letters within a row are significantly different ($P < 0.05$), $n = 3$.
$^{x–w}$ Different letters within a column with same color value are significantly different ($P < 0.05$).

Table 2
Effect of heat treatments condition and physical form on radical scavenging activity (%) of 70% ethanol extracts from whole (WGSE) and powder (PGSE) form of grape seeds extracts

Temperature	Heating time (min)								
	0	10	20	30	40	60	90	120	SEM
WGSE									
50	63.13^{cw}	68.66^{cx}	59.73^{ex}	71.98^{bx}	66.37^{dx}	70.94^{bx}	85.99^{aw}	70.43^{by}	0.46
100	63.13^{bw}	59.93^{cy}	60.07^{cx}	67.08^{by}	67.23^{bx}	76.31^{aw}	67.16^{by}	75.35^{ax}	0.52
150	68.13^{ew}	75.13^{cw}	64.94^{fw}	78.75^{bw}	83.91^{aw}	61.62^{gy}	72.84^{dx}	79.19^{bw}	0.48
200	68.13^{aw}	49.59^{bz}	44.65^{cy}	37.19^{dz}	29.08^{efy}	43.47^{cz}	28.34^{fz}	30.33^{ez}	0.49
SEM	1.00	0.46	0.28	0.34	0.38	0.53	0.72	0.64	
PGSE									
50	63.13^{cdw}	66.98^{dx}	63.47^{ey}	76.79^{awx}	72.51^{bx}	69.63^{cy}	72.43^{bx}	78.19^{aw}	0.53
100	63.13^{dw}	82.94^{aw}	70.09^{dx}	77.72^{bw}	78.66^{bw}	76.64^{bw}	77.26^{bw}	74.14^{cx}	0.71
150	68.13^{dw}	67.68^{dx}	74.84^{bw}	75.70^{ax}	77.73^{aw}	72.90^{cx}	52.26^{cy}	42.83^{fy}	0.62
200	68.13^{aw}	46.88^{cy}	43.30^{dz}	62.46^{by}	42.06^{dy}	36.06^{ez}	16.43^{fz}	13.94^{gz}	0.66
SEM	1.00	0.94	0.48	0.42	0.37	0.79	0.72	0.64	

$^{a–f}$ Different letters within a row are significantly different ($P < 0.05$), $n = 3$.
$^{x–w}$ Different letters within a column with same color value are significantly different ($P < 0.05$).

Table 3
Effect heat treatments condition and physical form on reducing power (Abs) of 70% ethanol extracts from whole (WGSE) and powder (PGSE) form of grape seeds extracts

Temperature	Heating time (min)								
	0	10	20	30	40	60	90	120	SEM
WGSE									
50	0.766^{bw}	0.713^{cx}	0.558^{fy}	0.707^{cx}	0.695^{cdy}	0.677^{dx}	0.852^{aw}	0.621^{ex}	0.006
100	0.766^{dw}	0.687^{fy}	0.669^{gx}	0.804^{cw}	0.791^{cx}	0.882^{aw}	0.702^{ex}	0.841^{bw}	0.005
150	0.766^{dw}	0.762^{dw}	0.816^{cw}	0.704^{ex}	0.865^{aw}	0.609^{gy}	0.685^{fc}	0.831^{bw}	0.003
200	0.766^{aw}	0.583^{bz}	0.395^{cz}	0.344^{ey}	0.255^{fz}	0.385^{dz}	0.233^{gz}	0.248^{fy}	0.003
SEM	0.004	0.004	0.003	0.005	0.002	0.006	0.004	0.006	
PGSE									
50	0.758^{cdw}	0.745^{cdx}	0.720^{ex}	0.790^{b}w	0.767^{bcx}	0.736^{ex}	0.775^{bcx}	0.839^{aw}	0.007
100	0.758^{dw}	0.822^{aw}	0.716^{ex}	0.775^{cwx}	0.784^{bcw}	0.794^{bw}	0.795^{bw}	0.748^{dx}	0.004
150	0.758^{bw}	0.691^{dy}	0.752^{bcw}	0.761^{bx}	0.779^{aw}	0.737^{cx}	0.399^{ey}	0.345^{fy}	0.005
200	0.758^{ax}	0.191^{cz}	0.178^{dy}	0.247^{by}	0.167^{dey}	0.159^{ey}	0.086^{fz}	0.083^{fz}	0.004
SEM	0.009	0.007	0.003	0.006	0.003	0.002	0.004	0.003	

$^{a–f}$ Different letters within a row are significantly different ($P < 0.05$), $n = 3$.
$^{x–w}$ Different letters within a column with same color value are significantly different ($P < 0.05$).

forms depend on species. Thus, effective processing step for liberating antioxidant compounds from different plant species may not be the same.

Radicals scavengers were evaluated by their reactivity toward a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH·). The radical scavenging activity

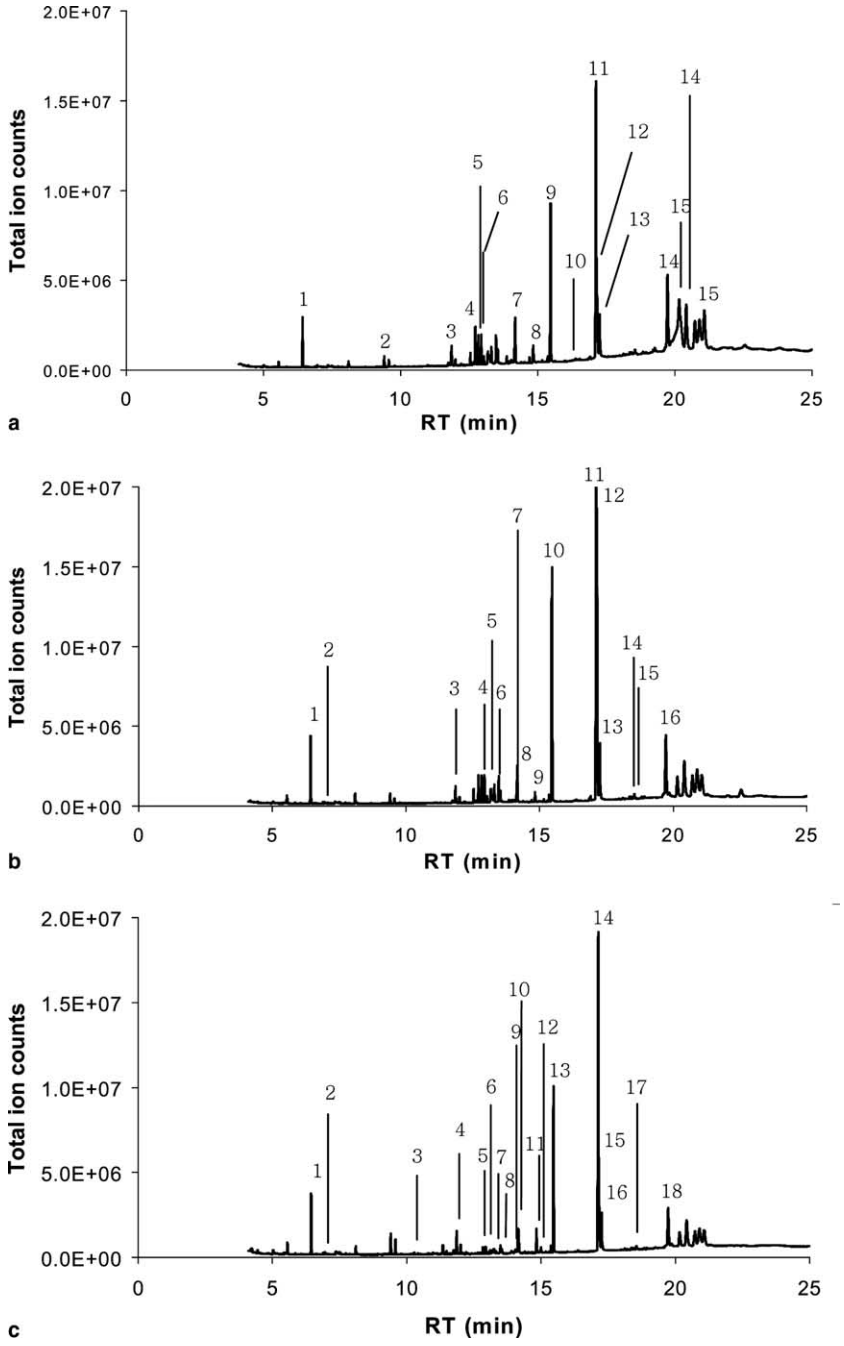

Fig. 1. Gas chromatography of 70% ethanol extracts of non-heated (a) and heated (b) at 100 °C for 10 min onto powered grape seed (PGSE), (c) at 150 °C for 40 min onto whole grape seed (WGSE). Peaks in (a) mean; 1, Glycerol; 2, Erythritol; 3, Xylitol; 4, α-Arabinofuranoside; 5, 1H-indole-2-carboxylic acid; 6, 2-Azathianthrene; 7, Glucopyranose; 8, 6-methylthio benzothieno quinoline; 9, Palmitic acid; 10, 2,2′-[(1-methyl-1,2-ethane)] phenol; 11, 4-amino-2-(2-quinolyl)-5H-[1] benzopyran [3,4–c] pyridin-5-one; 12, Linoleic acid; 13, Stearic acid; 14, Galactofuranose; 15, Arabinofuranose. Peaks in (b) mean; 1, Glycerol; 2, Trimethyl silanol benzoate; 3, Xylitol; 4, Arabinofuranose; 5, 2-Azathianthrene; 6, Galactofuranoside; 7, Mannitol; 8, Glucopyranose; 9, 1H-Indole-2-carboxylic acid; 10, Palmitic acid; 11, 4-amino-2-(2-quinolyl)-5H-[1] benzopyran [3,4–c] pyridin-5-one; 12, Linoleic acid; 13, Stearic acid; 14, Dibenzoazcyclodecane; 15, *N*-phenyl dibenzocarbazole; 16, Hexaethyldisloxane. Peaks in (c) mean; 1, Glycerol; 2, Trimethyl silanol benzoate; 3, 1,2,3-benzenetriyltris silane; 4, Xylitol; 5, Arabinofuranose; 6, 2-Azathianthrene; 7, Azelaic acid; 8, 3,4-dihydroxy benzoic acid; 9, Mannitol; 10, Glucitol; 11, 6-methylthio benzothieno quinoline; 12, *o*-Cinnamic acid; 13, Palmitic acid; 14, 4-amino-2-(2-quinolyl)-5H-[1] benzopyran [3,4–c] pyridin-5-one; 15, Linoleic acid; 16, Stearic acid; 17, Dibenzoazcyclodecane; 18, β-D-Galactofuranose.

Table 4
Effect of heat treatments condition and physical form of grape seed on catechins and caffeine contents of GSE, prepared from non-heated intact grape seed (IGSE); heated at 100 °C for 10 min onto powdered grape seed (PGSE); and heated at 150 °C for 40 min onto whole grape seed (WGSE)

	EC	ECG	EGC	EGCG	C	CG	GC	GCG	Caffeine
IGSE	0.56[a]	0.15[a]	0.96[a]	0.11[b]	0.13[b]	0.12[a]	0.02[c]	0.36[c]	0.69[b]
PGSE	0.66[a]	0.20[a]	0.86[a]	0.15[a]	0.16[a]	0.23[a]	0.12[a]	1.47[b]	0.96[a]
WGSE	0.38[a]	0.20[a]	0.87[a]	0.14[a]	0.14[a]	0.41[a]	0.12[b]	2.01[a]	0.74[b]
SEM	0.15	0.22	0.60	0.05	0.03	0.11	0.01	0.03	0.03

EC, epicatechin; ECG, epicatechingallate; EGC, epigallocatechin; EGCG, epigallocatechingallate; C, catechin; CG, catechingallate; GC, gallocatechin; GCG, gallocatechingallate. Each value was expressed as mg/mL of grape seed extracts.
[a–c] Different letters within a column are significantly different ($P < 0.05$), $n = 3$.
[d] Standard error of the means.

(RSA) of WGSE and PGSE were significantly increased by different heat treatments ($P < 0.05$) (Table 2). RSA of WGSE increased from 63.13% to 76.31% after treating them at 100 °C for 60 min, and to 83.91% after heat treatment at 150 °C for 40 min. While, RSA of PGSE increased to 78.19% after treating the powdered grape seeds at 50 °C for 120 min, and to 82.94% after treating them at 100 °C for 10 min. However, heating grape seeds at 200 °C decreased the RSA of WCPE and PGSE significantly ($P < 0.05$).

The power of certain antioxidants is associated with their reducing power (Jayaprakasha, Singh, & Sakariah, 2001). Duh (1998) reported that reducing properties of antioxidants are generally associated with the presence of reductions. The reducing power of GSE was significantly increased by different heat treatments ($P < 0.05$) (Table 3). The reducing power of WGSE increased from 0.766 (absorbance value) in control to 0.852 after heating the seeds at 50 °C for 90 min, and to 0.882 after heating the seeds at 100 °C for 60 min, and to 0.865 after heating them at 150 °C for 40 min. While the reducing power of PGSE increased to 0.839 with 50 °C heating for 120 min and to 0.822 with heating at 100 °C for 10 min. Also, heating the seeds at 200 °C decreased the reducing power of WCPE and PGSE significantly ($P < 0.05$).

3.2. Identification of grape seeds extracts

Phenolic acids and their derivatives are widely distributed in plants. A number of phenolic acids are linked to various cell wall components such as carbohydrates and proteins (Hartley, Morrison, Himmelsbach, & Borneman, 1990). Natural covalently bound low molecular weight phenolic compounds in plants have little antioxidant activities. However, the phenolic compounds liberated by far infrared heating or fermentation have strong antioxidant activities (Jeong, Kim, Park, & Lee, 2004c; Niwa, Kanoh, Kasama, & Negishi, 1998).

There are slight differences in the kinds of phenolic compounds detected in non-heated and heated (at 150 °C for 40 min) WGSE (Fig. 1). Several low molecular weight phenolic compounds such as azelaic acid, 3,4-dihydroxy benzoic acid and *o*-cinnamic acid were newly found in WGSE heated at 150 °C for 40 min. It was reported that benzoic and cinnamic acid derivatives of polyphenol compounds such as 3,4-dihydroxy benzoic acid and *o*-cinnamic acid can reduce ferrylmyoglobin (Natella, Nardini, Di Felice, & Scaccini, 1999) and inhibit oxidative modification of LDL by azoinitiators and metal catalysis (Laranjinha, Vieira, Madeira, & Almeida, 1995; Nardini et al., 1995). In addition, azelaic acid was reported to have antitumorual potential (Breathnach, 1999).

There were also significant differences in fatty acids. PGSE heated at 100 °C for 10 min showed 3.3- and 2.5-fold increased amount of linoleic acid (18:2) and palmitic acid (16:0), respectively, compared to those of non-heated control. Fatty acid also attribute antioxidant activity, especially palmitic acids were reported to be more effective free radical scavengers than β-carotene (Matsufuji, Nakamura, Chino, & Takeda, 1998).

3.3. Identification of catechins and caffeine of GSE

The contents of catechins and caffeine in GSE were significantly influenced by different heat treatment ($P < 0.05$) (Table 4). Especially, GCG of PGSE (heated at 100 °C for 60 min) and WGSE (heated at 150 °C for 40 min) increased from 0.36 mg/mL in non-heated control to 1.47 mg/mL, and to 2.01 mg/mL, respectively. Total catechin amount of the PSGE and the WGSE increased to 3.18 and 3.75 mg/mL, respectively, compared to 2.32 mg/mL of not-heated control. Caffeine of the PGSE also increased from 0.69 to 0.96 mg/mL after heating. Heating of green tea also increased the amount of catechins such as GCG, CG, GC in water extracts (Wang, Kim, & Lee, 2000). Our result showed that the contents of catechins and caffeine in GSE were also relevant to heating conditions and physical form of grape seeds.

4. Conclusions

Heat treatment of grape seeds liberated phenolic compounds, and thus increased the amounts of active

compounds in extracts. The condition of heating and physical form of grape seed affected antioxidant activity of grape seed extract. This study showed simple heating process can be used as a tool to increase antioxidant activity of GSE.

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