J Food Sci Nutr
Vol 9, p 144~149 (2004)

J Food Science and Nutrition

Changes in Functional Constituents of Grape (*Vitis vinifera*) Seed by Different Heat Pretreatments

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Abstract

Changes in functional constituents of grape (*Vitis vinifera*) seeds prepared by three different heat pretreatments were determined and compared with those of non-treated grape seed. The recovery of grape seed oils was generally increased by roasting, steaming and microwave processes, although the recovery of specific constituents varied among three heat pretreatments. The recovery of MeOH extracts of the seeds increased following the roasting process, whereas that of MeOH extracts decreased gradually with steaming and microwave treatments. Levels of four catechins in grape seeds: (+)-catechin, procyanidin B_2, (-)-epicatechin, and (-)-epicatechin gallate, were decreased with increased roasting and steaming time, but were unaffected by microwave treatment. During the three different heat pretreatments, levels and compositions of fatty acid did not change, whereas those of phytosterol compositions decreased greatly. These results suggest that a mild heat pretreatment, controlled for temperature and time, is needed to prevent a considerable loss in the level of valuable functional components in grape seed.

Key words: grape (*Vitis vinifera*) seed, heat pretreatments, functional constituents

INTRODUCTION

Grape (*Vitis vinifera*) seeds are a rich source of monomeric phenolic compounds, such as (+)-catechins, (-)-epicatechin and (-)-epicatechin 3-*O*-gallate, and their oligomeric procyanidins, which have been reported to have a variety of biological activities, including antioxidative (1-3), anti-atherosclerotic (4-6), anti-carcinogenic (7-9), anti-ulceric (10), and anti-cataractic (11) activities. Additionally, grape seeds are known to possess many phytochemical constituents such as linoleic acid, dietary fiber, tocopherol and phytosterols (12,13), although levels of all functional compounds varied by cultivar, maturation and processing (14-18).

At present, grape seed oil is widely used in many countries as a dietary supplement with antioxidative and antiatherosclerotic effects (19,20). Grape seed oils are traditionally prepared by a conventional method, which involves cleaning, roasting, grinding, and pressing processes, but not a refining process (21). Roasting grape seeds during the oil production was found to play important roles in the development of a pleasant aroma and taste (22,23), and to improve the recovery and functional components of the seed oils (24-26). Moreover, steaming and microwave processing along with the roasting process are currently employed as preliminary treatments to improve the physicochemical and nutritional quality of food products (27,28), and to increase the levels of one or more valuable phytochemicals in plant seeds (29-31). Thus, heat pretreatments, such as roasting, steaming, and microwave heating, have been reported to greatly influence on the yield and chemical composition of plant seeds. However, few studies have been conducted on the effects of heat pretreatments on the functional constituents of grape seeds.

The objective of this study was to investigate changes in catechins, fatty acid compositions and other phytochemical components, such as tocopherol and phytosterol, of grape seeds prepared with three different heat pretreatments including roasting, steaming and microwave heating.

MATERIALS AND METHODS

Materials and chemicals

Grape (*Vitis vinifera*) seed from Campbell Early grape was harvested in early September 2003 at the Modong farm, Sangju, Gyeongbuk, Korea. (+)-Catechin [(+)-C] and (-)-epicatechin [(-)-EC] were purchased from Fluka (Buchs, Switzerland). Procyanidin B_2 [PC-B_2, epicatechin-

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(4β→8)-epicatechin] and (-)-epicatechin gallate [(-)-ECg] were obtained from Iwai Chem. Co. (Tokyo, Japan). Four tocopherol isomers, three phytosterols (campesterol, stigmasterol, β-sitosterol), and free fatty acids were obtained from Sigma Chemical Co. (St. Louis, MO, USA). HPLC solvents were obtained from Merck (Darmstadt, Germany). All other reagents used for this study were of analytical grade.

Sample preparation

Grape seeds (50 g) were roasted in an electric roaster with constant stirring at 200°C for 5, 10, and 15 min. Meanwhile, another sample of the same seeds (50 g) was steamed in a domestic stainless steel steamer [dimensions 260 (W)×200 mm (H)] for 10, 30, and 60 min, and a third sample (50 g) was placed in a rotating glass container (dimensions 290 mm id) in the center of a domestic microwave (MW) oven (Samsung RE-C200T, frequency 2450 MHz, pulsed variable MW power output from 90 to 700 W by a timer, inner volume 21.8 L) and heated for 1, 3 and 5 min. The grape seeds preheated by three different heating methods were ground with a coffee maker and dried for 2 hr in a dry oven at 50°C before analysis of phytochemical constituents.

Preparation of oil and MeOH extract from grape seeds

To calculate the yields of oil and MeOH extract from grape seeds prepared by three different heat pretreatments, each ground seed (10 g) was extracted twice with $CHCl_3$-MeOH (2:1, v/v, 100 mL) for 2 hr in an ultrasonic cleaner (Bransonic 5210R-DTH, USA) at room temperature, filtered and evaporated under reduced pressure. The concentrated sample was redissolved again in *n*-hexane (10 mL) and filtered through a Whatman GF/A glass fiber filter (Whatman Laboratory Products, Clifton, NL, USA) to remove particles, and evaporated *in vacuo* to yield oil. Meanwhile, the defatted grape seed residues obtained above were extracted twice with 80% aq. MeOH (100 mL) for 2 hr under reflux, filtered and evaporated *in vacuo* to obtain MeOH extracts.

Catechin analysis

Four catechins, (+)-C, (-)-EC, PC-B_2 and ECg, in grape seed during heat pretreatments were determined by HPLC as previously described (32). Each MeOH extract (0.2 g) obtained previously was solubilized in 80% aq. MeOH (10 mL) and passed through a 0.45 μm membrane filter (Gelman, Ann Arbor, MI, USA) and injected in HPLC for quantification of the four catechins. HPLC analysis was performed on an HPLC system (Gilson 506B, Middleton, WI, USA) equipped with 170 UV-VIS detector, Gilson Unipoint™ 3.0 software and 231XL autosampler with a 10 μL loop. A YMC-Pack Pro C_{18} column (5 μm, 4.6 ID×250 mm, YMC Inc, Milford, MA, USA) at a flow rate of 1.0 mL/min with UV detector at 280 nm. A mobile phase eluted gradiently from solvent A (4.5% formic acid in H_2O) to solvent B (90% CH_3CN containing 10% solvent A) for 50 min.

Analysis of fatty acid composition

Each grape seed oil (100 mg) obtained previously was placed in a tube (10 mL) with screw cap and solubilized with 6% H_2SO_4 in MeOH (3 mL) and then heptadecanoic acid (10 μL, 1 mg/mL in hexane) as an internal standard was added. The mixture was vortexed vigorously and esterified for 1 hr in a dry oven at 70°C. Methyl esters of FA were extracted with hexane and then dehydrated with anhydrous Na_2SO_4. The aliquots (1 μL) of the extracts were injected into a gas chromatography (Hewlett-Packard 6890 series, USA) equipped with a FID. The column used was a Supelcowax™-10 fused-silica capillary column (60 m×0.25 mm ID; Supelco, Bellefonte, PA, USA). The carrier gas was helium, and the total gas flow rate in inlet was 52.5 mL/min (constant flow mode) with split mode (50:1). The injector, oven, and detector temperatures were 250°C, 190°C and 260°C, respectively.

Analysis of phytosterols

Quantitative analysis of the three phytosterols in grape seed oil was performed with the obtained external standard curve as previously reported (33). Grape seed oil (0.1 g) obtained above was placed into a test tube (25 mL) with a screw cap and then redissolved in 2 N KOH in EtOH (2 mL). The sample was saponified for 15 min in a water bath at 100°C and cooled in an ice bath. Two mL each of water and hexane was added and shaken gently, and the upper layer was dehydrated with anhydrous Na_2SO_4. Aliquots (1 μL) of the extracts adding 5-cholesterol (1 mg/1 mL in *n*-hexane, 100 μL) as an internal standard were injected into a gas chromatography (Hewlett-Packard 6890 series, Avondale, PA, USA) equipped with a FID. The column used was a Ultra 2 fused-silica capillary column (60 m×0.25 mm ID; Hewlett-Packard, Avondale, PA, USA) and the carrier gas was helium (25 mL/min). The injector, oven, and detector temperatures were 300°C, 285°C and 300°C, respectively.

Analysis of tocopherols

Quantitative analysis of four tocopherol isomers in grape seed oil was carried out according to the AOCS method reported previously (33). Grape seed oil (0.1 g) was solubilized in hexane (10 mL) and passed through a PTFE syringe filter (25 mm 0.2 μm, Whatman, Clifton, NJ, USA) and evaporated under reduced pressure. The

concentrated sample was solubilized in *n*-hexane and injected into a liquid chromatography (LC) to quantify four tocopherols (α-, β-, γ- and δ-tocopherols). The LC system consisted of an HPLC (Younglin Acme, Seoul, Korea) injector with a 10 μL sample loop and a UV detector (Younglin Absorbance, Seoul, Korea) at 295 nm. A LiChrosorb DIOL column (5 μm, 3×100 mm, Merck Co, Chrompack, Palo Alto, CA, USA) was used. The mobile phase was the mixture of *n*-hexane and acetic acid (1000:1, v/v) at 0.5 mL/min.

For quantitative analysis of functional constituents in grape seeds, each heat pretreatment was repeated twice with duplicate samples, and the data presented are means ±standard deviation.

RESULTS AND DISCUSSION

Yield of oil and MeOH extract from heat-pretreated grape seeds

The yields (%) of oils and MeOH extracts from grape seeds prepared by three different heat pretreatments; roasting, steaming and microwave heating, are shown in Table 1. The yield of oil increased progressively up to ~13% and ~6% for steaming and microwave heating, respectively, as compared to the control, while that of oil for roasting process increased up to ~8% for 5 min of heat treatment and then decreased to ~3% for 10 min. In contrast, the yield of MeOH extract from roasted seeds increased progressively up to 5%, whereas for steamed seeds it decreased progressively up to 19%. Particularly, microwave heating caused a considerable increase up to 17% in the yield of MeOH extracts for 1 min, and then decreased slightly to ~8% for 5 min.

Table 1. Yield of oils and MeOH extracts from grape seed prepared by three different heat pretreatments

Sample	Yield (%, dried grape seed)	
	Oil	MeOH ext.
Control (no heat treatment)	7.37±0.53	4.50±0.13
Roasting time (min)		
3	7.31±0.43[1] (99.2)[2]	4.45±0.14 (98.9)
5	7.94±0.64 (107.7)	4.52±0.27 (100.4)
10	7.58±0.28 (102.9)	4.73±0.32 (105.1)
Steamming time (min)		
10	7.69±0.34 (104.3)	4.55±0.24 (101.1)
30	7.82±0.41 (106.1)	4.25±0.16 (94.4)
60	8.31±0.54 (112.8)	3.66±0.10 (81.3)
Microwave time (min)		
1	7.57±0.33 (102.7)	5.26±0.43 (116.9)
3	7.77±0.36 (105.4)	4.94±0.34 (109.8)
5	7.79±0.52 (105.7)	4.88±0.31 (108.4)

[1]Values are mean±SD of duplicate analyses.
[2]% change.

Thus, three heat pretreatments resulted in some small changes in the yield of oil and MeOH extract from grape seed. Organoleptic observation revealed that a pleasant aroma or taste of the grape seed developed during the roasting processing for 10 min and microwave treatment for 5 min, but roasting for 20 min resulted in the production of extensive charring, and very low yields (<20%) of grape seed oil and MeOH extract (data not shown). Additionally, the yields of oil and MeOH extract from the grape seeds used in this study were considerably lower than those of grape seeds harvested in September 2002, indicating that the yields of oil and MeOH extract of grape seeds could be affected by maturity, genotype and processing (14,16,18).

Catechin composition

Changes in level of four catechins, such as (+)-C, (-)-EC, PC-B_2 and ECg, which are the predominant phenolic compounds in grape seeds, were determined by HPLC in relation to three different heat pretreatments (Table 2). During the roasting and steaming processes, concentrations of the four catechins decreased considerably with increased roasting and steaming time. During the microwave treatment, there was a slight increase in levels of two catechins, but not for PC-B_2 and ECg of grape seeds. Thus, roasting and steaming processes caused a considerable decrease in concentrations of four catechins, which are very susceptible to oxidation (34).

FA composition

The effect of heat pretreatments on the fatty acids (FA) composition of grape seeds is shown in Table 3. Grape seed oil (non-treated) consisted of 0.10% myristic acid, 10.2% palmitic acid, 0.20% palmitoleic acid, 2.0% stearic acid, 22.5% oleic acid, 64.0% linoleic acid, and 0.4% linolenic acid. Following the three different heat pretreatments, there were no differences in FA composition of grape seed oils. A similar trend has been reported for the FA composition of corn fiber and rice germ oils following heat pretreatments including roasting and microwave processes (25,30).

Phytosterol and tocopherol composition

Changes in the concentrations of three phytosterols in grape seed oils prepared by three different heat pretreatments are shown in Table 4. Three phytosterol derivatives, campesterol, stigmasterol and β-sitosterol, were identified, of which β-sitosterol was the predominant phytosterol component.

Grape seed oil (non-treated) had 20.42 mg% campesterol, 15.16 mg% stigmasterol, and 116.03 mg% β-sitosterol. With three types of heat pretreatments, the levels of three phytosterols decreased progressively with

Table 2. Changes in the concentrations of four catechins in grape seed prepared by three different heat pretreatments

Treatment	Catechins (%, grape seed)				
	(+)-Catechin	Procyanidin B_2	(-)-Epicatechin	(-)-Epicatechin gallate	Total catechin[1)]
Control (no heat treatment)	0.627±0.030[2)]	0.047±0.003	0.507±0.023	0.031±0.001	1.212±0.076
Roasting time (min)					
3	0.581±0.020	0.032±0.013	0.478±0.024	0.027±0.002	1.118±0.068
5	0.579±0.016	0.029±0.007	0.456±0.023	0.028±0.001	1.092±0.053
10	0.334±0.017	0.022±0.009	0.229±0.020	0.016±0.001	0.601±0.028
Steamming time (min)					
10	0.583±0.008	0.039±0.002	0.487±0.008	0.028±0.002	1.137±0.019
30	0.524±0.011	0.027±0.004	0.431±0.013	0.020±0.001	1.002±0.024
60	0.385±0.015	0.022±0.003	0.387±0.013	0.014±0.001	0.808±0.032
Microwave time (min)					
1	0.623±0.012	0.043±0.007	0.498±0.015	0.033±0.001	1.197±0.026
3	0.627±0.023	0.042±0.005	0.521±0.017	0.034±0.002	1.224±0.050
5	0.693±0.018	0.041±0.008	0.582±0.017	0.034±0.002	1.350±0.031

[1)](+)-Catechin + procyanidin B_2 + (-)-epicatechin + (-)-epicatechin gallate.
[2)]Values are mean±SD of duplicate analyses.

Table 3. Changes in the fatty acid composition of grape seed oil prepared by three different heat pretreatments

Treatment	Fatty acids (Mol %)						
	Myristic acid (C_{14})	Palmitic acid ($C_{16:0}$)	Palmitoleic acid ($C_{16:1}$)	Stearic acid ($C_{18:0}$)	Oleic acid ($C_{18:1}$)	Linoleic acid ($C_{18:2}$)	Linolenic acid ($C_{18:3}$)
Control (no heat treatment)	0.1±0.1[1)]	10.2±0.8	0.2±0.1	3.0±0.1	22.5±0.1	64.0±0.9	0.4±0.1
Roasting time (min)							
3	0.1±0.0	10.5±0.4	0.2±0.0	3.1±0.1	22.5±0.4	63.2±0.0	0.5±0.0
5	0.1±0.1	10.0±0.7	0.2±0.0	3.1±0.1	22.8±0.1	63.5±0.8	0.5±0.1
10	0.1±0.0	10.3±0.1	0.2±0.0	3.1±0.1	23.0±0.1	62.8±0.0	0.6±0.1
Steamming time (min)							
10	0.1±0.0	9.9±0.1	0.2±0.0	3.0±0.0	22.3±0.1	64.1±0.3	0.5±0.1
30	1.2±0.3	10.3±0.3	0.3±0.0	2.9±0.0	22.2±0.5	62.5±0.2	0.5±0.0
60	1.3±0.1	10.2±0.3	0.3±0.0	3.0±0.1	21.9±0.0	62.9±0.4	0.5±0.0
Microwave time (min)							
1	1.2±0.2	10.1±0.0	0.3±0.0	3.1±0.1	22.0±0.1	62.8±0.6	0.5±0.1
3	1.4±0.1	10.8±0.8	0.4±0.1	3.3±0.2	22.3±0.0	61.4±1.2	0.5±0.1
5	0.7±0.1	10.1±0.9	0.5±0.1	2.6±0.3	22.0±0.9	62.6±2.6	1.5±1.1

[1)]Values are mean±SD of duplicate analyses.

increases in treatment time, with the exception of campesterol for the roasting process. Levels of total phytosterol for roasting, steaming and microwave heating decreased progressively up to totals of ~72%, ~23% and ~37%, respectively, as compared to the control. Particularly, there was a significant decrease in the content of total phytosterol in steamed and microwaved seeds, but much less loss for roasted seeds. Moreau et al. (30) offered a possible explanation for the heat-induced decrease in the levels of free phytosterols in corn fiber oil, suggesting that free phytosterols evaporates easily under vacuum and high temperature due to their low boiling points. Therefore, development of suitable processing technology is needed to retain or enhance the levels of phytochemicals in grape seed oil without removing significant amounts of valuable cholesterol-lowering phytosterol components (35).

Four tocopherol isomers were not found in grape seeds in this study, regradless of heat pretreatments, or lack thereof (data not shown). In contrast to this experiment, Kinsella (12) reported that grape seeds contained a large amount of α-tocopherol, suggesting that levels of tocopherol in grape seeds varies among cultivar and maturation. Previously, several studies (24,25,29,30) reported that heat pretreatments resulted in significant increases or decreases in tocopherol content, which is not con-

Table 4. Changes in phytosterol content of grape seed oil prepared by three different heat pretreatments

Treatment	Phytosterols (mg%, grape seed oil)			
	Campesterol	Stigmasterol	β-Sitosterol	Total sterol[1)]
Control (no heat treatment)	20.42±1.45[2)]	15.16±0.54	116.03±3.53	151.61±2.42
Roasting time (min)				
3	13.23±0.53	15.31±0.56	104.52±3.10	133.06±2.31
5	14.61±0.57	12.71±0.34	101.62±2.43	128.94±1.83
10	14.63±0.36	8.42±0.11	85.35±1.13	108.40±0.72
Steamming time (min)				
10	13.34±0.31	8.33±0.24	77.63±1.31	99.30±0.74
30	9.53±0.24	7.73±0.12	66.82±0.87	84.08±0.53
60	ND[3)]	ND	34.53±1.03	34.53±1.03
Microwave time (min)				
1	8.54±0.13	8.02±0.42	62.72±0.52	79.28±0.53
3	6.53±0.24	7.83±0.32	57.43±1.23	71.79±0.63
5	ND	ND	55.53±0.92	55.53±0.92

[1)]Campesterol + stigmasterol + β-sitosterol.
[2)]Values are mean±SD of duplicate analyses.
[3)]ND: Not detected.

sistent with our results. Previous study recommended that a saponification step is often required to release bound tocopherol as part of the routine extraction of total tocopherol (36). However, we found that saponification of grape seed oil by alkaline hydrolysis was not helpful to quantify tocopherol isomers in grape seed oil.

In conclusion, the heat pretreatments, such as roasting, steaming and microwave heating, have a positive effect on recovery of oils and MeOH extracts from grape seeds. However, a modest reduction in levels of catechins and phytosterols in grape seeds was observed following the three different heat pretreatments, but fatty acid and tocopherol compositions were not affected. Therefore, a mild heat pretreatment, controlling for temperature and time, is needed to prevent a considerable loss of the level of valuable functional components in grape seeds. This study is the first report on chemical changes in grape seeds with different heat pretreatments including roasting, steaming and microwave processing.

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(Received April 27, 2004; Accepted June 4, 2004)