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Tocols in caneberry seed oils

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ABSTRACT

The compositional analysis of tocols in oils extracted from Korean caneberry seeds was compared with commercial soybean, corn, olive, canola, perilla, and grape seed oils. The oils from caneberry seeds of six different species were extracted using either a chloroform–methanol–water system or hot hexane. Tocols from all of the oils were analysed using isocratic HPLC. The contents of total tocopherols in the caneberry seed oils were about 75–290 mg/100 g oil, whereas tocotrienols were not detected. γ-Tocopherol was the most abundant tocopherol (31.8–239 mg/100 g oil) in the caneberry seed oils, followed by α-tocopherol (7.6–58.2 mg/100 g oil). The contents of total tocols in soybean, corn, olive, canola, perilla, and grape seed oils were 99.9, 61.1, 28, 27, 45.4, and 52.2 mg/100 g oil, respectively. Total tocol content was higher in most of the caneberry seed oils including the refined ones than in the commercial vegetable oils.

1. Introduction

Increased interest in the beneficial health effects of caneberries has boosted the production of caneberries (raspberries) in Korea in recent years. Korea produced 1840 metric tons of caneberries in 2004 and about half was used for wine making (personal communication from the Agricultural Technology Service Center, Gochang, Korea). Pomace from wine production contains pulp and seeds and has been mostly used for fertiliser or animal feed. The seeds are known to have a considerable amount of oil and their potential as specialty nutraceutical oil was suggested based on a high level of unsaturation (Bushman et al., 2004; Johansson, Laakso, & Kallio, 1997; Oh, Hwang, Shin, Lee, & Kim, 2007) and a high content of tocols (Oomah, Ladet, Godfrey, Liang, & Girard, 2000; Pourrat & Pourrat, 1973).

According to previous reports, the seeds of different types of caneberries constituted 4–12% (wet weight basis) of the fruit (Johansson et al., 1997; Oh et al., 2007), and contained 10–28% oil (dry weight basis) (Bushman et al., 2004; Johansson et al., 1997; Oh et al., 2007). The major fatty acids in the caneberry seed oils were linoleic (41–70%), linolenic (13–36%), and oleic (11–19%) acids (Bushman et al., 2004; Johansson et al., 1997; Oh et al., 2007). Oh et al. (2007) reported that all of the oils from Korean caneberry seeds contained more than 90% unsaturated fatty acids.

Tocols (tocopherols and tocotrienols) are a group of lipid soluble compounds generally called vitamin E. Naturally occurring vitamin E is composed of eight vitamers: α-, β-, γ-, and δ-tocopherols and the corresponding unsaturated tocotrienols. It is well-known that tocols have various health benefits (Campbell, Stone, Whaley, & Krishna, 2003; Hosomi et al., 1997; Jiang, Christen, Shigenaga, & Ames, 2001; Mishima et al., 2003; Panfili, Fratianni, & Irano, 2003; Saldeen, Li, & Mehta, 1999; Sen, Khana, & Roy, 2006). Tocols are a major primary antioxidant group present in vegetable oils. The main sources of tocols in the human diet are vegetable oils, fruits, seeds, nuts and cereals, and products derived from them (Morphy, Subar, & Block, 1990; Ryynänen, Lampi, Salo-Väänänen, Ollilainen, & Piironen, 2004).

Oomah et al. (2000) reported that raspberry seed oils contained about six times higher tocopherols than those in safflower and grape seed oils. According to their report, raspberry seed oils were fairly stable to oxidation, although the oils were highly unsaturated. Oils from raspberry seeds were reported to have excellent anti-inflammatory activity compared to virgin avocado, grape seed, and wheat germ oils (Pourrat & Pourrat, 1973).

Since the oils from Korean caneberry seeds have been shown to have an excellent fatty acid composition (Oh et al., 2007), supplementary data for the tocol content in the oils might suggest

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additional benefits for the development of these oils as a specialty nutraceutical item. This study was done to analyse the tocols in oils extracted from Korean caneberry seed cultivars in comparison with commercial vegetable oils.

2. Materials and methods

2.1. Materials

Different types of caneberries harvested in Korea were used: black raspberries (*Rubus occidentalis*) (cultivated) from farmers in Gochang and Goseong Counties and from the Raspberry Experiment Station, Agricultural Technology Service Center in Gochang; red raspberries (*Rubus idaeus*) (cultivated) from the Raspberry Experiment Station; mountain raspberries (*Rubus crataegifolius*) (cultivated) from a farmer in Goseong; Korean raspberries (*Rubus coreanus*) (wild), golden Korean raspberries (cultivated), boysenberries (cultivated) and thornless blackberries (*Rubus fruticosus*) (cultivated) from a farmer in Wanju County. The caneberries were stored at −18 °C and used within 2 months. Soybean (Ottogi (Anyang, Korea), CJ Cheiljedang (Seoul, Korea), and Sajo O&F (Seoul, Korea)), corn (Daesang (Seoul, Korea), CJ Cheiljedang, and Sajo O&F), olive (Daesang, CJ Cheiljedang, and Fontana (Seoul, Korea)), canola (Sajo O&F, Dongwon F&B (Seoul, Korea), and Canbra Foods (Lethbridge, Alberta, Canada)), perilla (Saimdang Food (Paju, Korea)) and grape seed oils (Ottogi, Daesang, and CJ Cheiljedang) were purchased from a local market in Jeonju, Korea. Using hot hexane two other perilla oils were extracted in the laboratory from Korea-origin perilla and China-origin perilla. All these vegetable oils were stored at −18 °C and used within 2 months.

2.2. Reagents

Standard α-tocopherol was purchased from Sigma (St. Louis, MO), β-, γ-, and δ-tocopherols from Supelco (Bellefonte, PA), and tocotrienols from Davos Life Science (Singapore).

2.3. Separation of seeds from caneberries

Seeds from the caneberries were separated as in a previous report (Oh et al., 2007). The berry fruits (about 10–15 kg each time) were crushed by hand in cold tap water and soaked in a 10-l container of water for approximately 24 h. The pulp suspended in the water was rubbed between the palms of the hands separating residual berry pulp from the seeds. Seeds either initially or after the second crushing sank to the bottom of the container. After seed settling, the mixture of pulp and water was decanted off. Pulp remaining on the seeds were washed off with tap water until the washed water was clear. Excess water was drained off from the seeds using a screen through which the seeds did not go. Following the final washing, the seeds were spread onto cotton gauze to dry at room temperature for about 24 h and then stored in a plastic bag at room temperature. All analyses were completed within 15 days.

2.4. Oil extraction from caneberry seeds for analyzing tocols in the seeds

Oil was extracted according to the Bligh and Dyer (1959) method. Seeds were ground using a mortar. The ground seed (30 g) was weighed into a beaker. It was blended for 30 s using a homogeniser (M133/1281-0; Biospec Products, Inc., Bartlesville, OK) with a mixture of 50 ml chloroform, 100 ml methanol and 50 ml 0.88% KCl solution (all reagent grade from Oriental Chemical Inc., Seoul, Korea). Fifty millilitres chloroform and 40 ml 0.88% KCl solution were added and blended for one more min. The blended mixture was transferred to a 250 ml centrifuge bottle and centrifuged for 20 min at 2325*g* (Supra 25K; Hanil Science Industrial Co., Incheon, Korea). The supernatant was transferred to a separating funnel and allowed to settle for 40 min. The chloroform layer was separated through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone), on which about 10 g anhydrous Na_2SO_4 was placed. Chloroform was evaporated using an Eyela rotary vacuum evaporator (N-N; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) connected to an Eyela aspirator (A-3S). The extracted oil was flushed with nitrogen and stored at −40 °C until used.

2.5. Oil extraction from caneberry seeds using hot hexane

Only the seeds from black raspberries were pooled for oil extraction using hot hexane. Seeds were ground using a mortar. Ground seeds (500–600 g for each batch) and hexane (Samchun Chemical, Pyeongtaek, Korea) (2 volumes of seed weight) were put into a round-bottomed flask connected to a condenser. The flask containing seeds and hexane was heated and refluxed for 2 h. The contents were filtered through Whatman No. 2 filter paper. The filtered residue was put back into the flask with additional hexane (1.5 × volume of the original seed weight). The content was then twice refluxed for 2 h and filtered as mentioned above. The filtrates were collected and evaporated using the vacuum rotary evaporator. The crude oil was collected and flushed with nitrogen and stored at −40 °C until used.

2.6. Refining of hexane-extracted crude oil

The crude oil (about 1000 g) extracted using hot hexane was put into a round-bottomed flask. Phosphoric acid (Showa Chemical, Tokyo, Japan) solution (10%, 20 ml) was added. The content were heated at 75–80 °C for 30 min and cooled to room temperature. They were then centrifuged at 1780*g* for 20 min. Degummed oil was collected. Based on the acidity of the degummed oil, it was neutralised by adding 8% NaOH (Samchun Chemical) solution and washing with 7.5% water. The neutralized oil was bleached using a mixture of activated clay (powder type, food grade; Dae Il Chemical, Pohang, Korea) and activated carbon (Shin Ki Chemical, Yangsan, Korea) (2:1, added at 2%). The bleach mixture was added at 75 °C and the oil was heated to 110 °C under 50 mm Hg, and held for 15 min while swirling. The oil was centrifuged to collect the bleached oil. The bleached oil was deodorised using a device assembled in the lab. The oil was heated to 240 °C under 3–5 mm Hg with steam blowing from a flask with boiling water for 2 h.

2.7. Saponification of oils for tocol analysis

Tocols were extracted from the oils according to a previous report (Kim et al., 2002). The oil (1.0 g) was mixed with ethanolic pyrogallol (5%, 4 ml) (Mallinckrodt Specialty Chemicals Co., Chesterfield, MO) in a 250 ml round-bottomed flask containing a few boiling chips and fitted with a reflux condenser and heated. KOH solution (50%, 1.5 ml) was added when the mixture began to boil and the boiling was continued for 5 min. The flask was placed in an ice bath, and 20 ml water and 30 ml diethyl ether (Fisher Scientific, Fair Lawn, NJ) were added. The mixture was transferred to a 250 ml separating funnel and kept until the two layers were clearly separated. The upper layer was collected. The extraction process with 30 ml diethyl ether was repeated twice more. The collected upper layers were washed three times with 30 ml water and filtered through Whatman No. 2 filter paper with anhydrous Na_2SO_4 (about 4 g) on it and evaporated using the vacuum rotary evaporator (at 30 °C) to complete dryness. The residue was diluted with 10 ml hexane and filtered through a Millipore 0.45 μm FH membrane (Millipore Corporation, Carrigtwohill, Ireland).

2.8. Analysis of tocols using HPLC

Tocols were analysed (Kim et al., 2002) using an isocratic HPLC (Waters 510; Waters Corp., Milford, MA) equipped with a Lichrospher Si-60 column (250 mm length × 4.6 mm i.d.; Merck Co., Darmstadt, Germany). The detector was an Alltech Evaporative Light Scattering Detector 800 (Deerfield, IL), operated at 40 °C under a constant flow of nitrogen (3.0 bar). The HPLC was operated in isocratic mode at a flow rate of 1 ml/min. The mobile phase was hexane/iso-propanol (J.T. Baker, Phillipsburg, NJ) (99:1, v/v). The standard tocols were diluted to concentrations of 0.002–2.5 μg/20 μl in hexane for the standard curves. Samples were prepared in hexane (2000 μg/20 μl), and 20 μl of each sample was injected for analysis. To improve the stability and reproducibility of the silica column, after eight injections the column was reactivated with a solution of 10% isopropanol in hexane (v/v) (Panfili et al., 2003).

2.9. Statistical analysis

The seeds collected from each caneberry sample were used for triplicate extractions of oils and analyses of tocols. Means of tocol levels were statistically analysed for differences among the caneberry samples using ANOVA and Duncan's multiple range tests ($\alpha = 0.05$) (SPSS 12.0KO for Windows, Release 12.0.1, SPSS Inc., Chicago, IL). Hot hexane extraction and refining of black raspberry seed oil was done in triplicate. Means of tocols in each commercial vegetable oil were obtained from three different brands with three measurements for each brand. ANOVA and Duncan's multiple range tests ($\alpha = 0.05$) were done among the means of all tocol samples.

3. Results and discussion

The compositions of tocols in caneberry seed oils and commercial vegetable oils analysed using HPLC are summarised in Tables 1 and 2. Total tocopherol contents in caneberry seed oils were quite variable among the species of caneberries, ranging from 75.2 mg/100 g oil in red raspberry seed oil to 290 mg/100 g oil in golden Korean raspberry seed oil (Table 1). The oil extracted from the seeds in black raspberry, which are the most widely cultivated in Korea, contained 139–181 mg tocopherols/100 g oil. α-Tocopherol and γ-tocopherol were detected in all the caneberry seed oils. The major tocopherol was γ-tocopherol (31.8–239 mg/100 g oil), followed by α-tocopherol (7.6–58.2 mg/100 g oil). Golden Korean raspberry seed oil contained the highest amount of γ-tocopherol (239 mg/100 g oil), followed by thornless black raspberry seed oil (142 mg/100 g oil). One of the black raspberry cultivars had the largest amount of α-tocopherol (58.2 mg/100 g oil). Tocotrienols were not detected in any of the caneberry seed oils.

The commercial soybean and olive oils contained all the tocopherols (Table 2). The soybean oils contained the highest levels of total tocols (99.9 mg/100 g oil) among the tested commercial vegetable oils. Tocotrienols were not detected in the soybean oils. Corn oils contained the second largest amounts of total tocols (61.1 mg/100 g oil). It should be noted that the grape seed oils contained fairly high amounts of α- and γ-tocotrienols compared to the other oils.

Caneberry seed oils contained higher amounts of total tocols than any of the vegetable oils, except for the red raspberry seed oil (Tables 1 and 2). Since the data presented in Table 1 are the values for tocopherols using the chloroform–methanol–water system, another extraction was employed using hot hexane, which was more likely to have been used with the commercial vegetable oils. For this extraction only the seeds from black raspberries were examined. The levels of α- and γ-tocopherols in the oils extracted using hot hexane were a little higher than from the chloroform–methanol–water system but this difference was not statistically significant ($\alpha = 0.05$). Refining of the oils extracted using hot hexane significantly reduced the tocopherol levels ($\alpha = 0.05$). The refined oil still contained significantly larger amounts of α, γ, and total tocopherols than the commercial oils.

Reviewing previous studies of the tocol contents in caneberry seed oils, Oomah et al. (2000) reported that the oil extracted from raspberry seeds using hexane contained 71 mg α-tocopherol/100 g oil, 272 mg γ-tocopherol/100 g, and 17 mg δ-tocopherol/100 g. These values are higher than those that we observed. Total tocopherol levels reported by Bushman et al. (2004), who analysed the tocopherol levels in five different kinds of caneberry seed oils, were 73–177 mg/100 g oil, which were similar to our values.

Tocopherol levels in vegetable oils reported by other researchers were also mostly less than those in caneberry seed oils. Lee and Lee (2006) and Ahmed, Daun, and Przybylski (2005) reported that soybean oil contained 5–20 mg α-tocopherol/100 g oil, 39–69 mg γ-tocopherol/100 g, and 21–46 mg δ-tocopherol/100 g; Lee and Lee (2006) and Cunha, Amaral, Fernandes, and Oliveira (2006) reported that olive oil contained 9–26 mg α-tocopherol/100 g oil and less than 1 mg each of the other minor tocols/100 g oil. Tocol contents in the caneberry seed oils were comparably higher than those in the other vegetable oils, too (Lee & Lee, 2006).

The fairly large amounts of tocopherols in caneberry seed oils could explain their oxidation stability (Oomah et al., 2000) despite their unsaturation level of more than 90% (Oh et al., 2007). The excellent anti-inflammatory activity of caneberry seed oil (Pourrat & Pourrat, 1973) might be also attributed to their high content of

Table 1
Contents of tocols in the oils of seeds from Korean caneberries (mg/100 g oil)[i]

Caneberry[k]	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherols
Black raspberry – A-I	50.0 ± 2.2[a]	ND[j]	89.3 ± 4.4[a]	ND	139 ± 4.0[a]
Black raspberry – A-II	58.2 ± 10.6[a]	ND	114 ± 11.4[bc]	ND	172 ± 8.9[bc]
Black raspberry – B	35.9 ± 3.2[bc]	ND	128 ± 2.2[de]	17.0 ± 3.0[a]	181 ± 2.2[b]
Black raspberry – D	50.2 ± 4.6[a]	ND	105 ± 4.2[b]	15.5 ± 2.1[a]	171 ± 7.6[bc]
Red raspberry – D	28.0 ± 0.6[c]	2.8 ± 2.5[a]	31.8 ± 0.6[f]	12.6 ± 1.0[b]	75.1 ± 0.6[d]
Mountain raspberry – B	8.8 ± 1.9[d]	ND	119 ± 4.3[cd]	ND	128 ± 2.5[e]
Korean raspberry – C	41.3 ± 3.3[b]	2.4 ± 2.1[a]	111 ± 5.0[bc]	9.0 ± 0.8[c]	163 ± 4.5[c]
Golden Korean raspberry – C	38.6 ± 7.5[b]	ND	239 ± 8.1[g]	12.1 ± 1.3[b]	290 ± 13.2[f]
Boysenberry – C	13.2 ± 1.4[d]	ND	137 ± 7.9[eh]	ND	150 ± 6.6[ag]
Thornless blackberry – C	7.6 ± 0.6[d]	ND	142 ± 5.0[h]	10.9 ± 1.1[bc]	160 ± 4.8[cg]

[a–h] Samples with the same letter in a column are not significantly different ($\alpha = 0.05$).
[i] All data are mean value ± standard deviation of three measurements; Tocotrienols were not detected.
[j] ND: not detected.
[k] A-I and A-II: from two farmers in Gochang; B: from a farmer in Goseong; C: from a farmer in Wanju; D: from the Raspberry Experiment Station, Agricultural Technology Service Center in Gochang.

Table 2
Contents of tocols in black raspberry seed oils and commercial vegetable oils (mg/100 g oil)

Samples	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	α-Tocotrienol	γ-Tocotrienol	Total tocols
Black raspberry seed oil[f]	48.6 ± 9.3^{ab}	ND[k]	109 ± 16.2^{ab}	8.2 ± 9.4^{ac}	ND	ND	166 ± 18.2^{a}
Black raspberry seed oil – hot hexane-extracted[g]	50.9 ± 3.0^{a}	ND	113.7 ± 8.3^{a}	11.1 ± 3.5^{a}	ND	ND	175 ± 7.1^{a}
Black raspberry seed oil – hot hexane-extracted and refined[h]	42.6 ± 3.2^{b}	ND	95.2 ± 5.3^{b}	3.9 ± 0.2^{acd}	ND	ND	142 ± 3.1^{b}
Soybean oil[i]	17.3 ± 3.4^{c}	6.9 ± 3.8^{a}	57.1 ± 8.7^{c}	18.6 ± 4.0^{b}	ND	ND	99.9 ± 16.5^{c}
Corn oil[i]	17.3 ± 1.5^{c}	ND	42.4 ± 7.6^{d}	ND	0.7 ± 1.2^{a}	ND	61.1 ± 8.2^{d}
Olive oil[i]	15.8 ± 1.2^{c}	1.5 ± 0.5^{bc}	2.1 ± 0.4^{e}	1.9 ± 0.6^{cd}	6.8 ± 1.3^{b}	ND	28.0 ± 3.2^{e}
Canola oil[i]	11.2 ± 6.0^{c}	2.5 ± 1.6^{bc}	13.2 ± 4.5^{e}	ND	ND	ND	27.0 ± 12.1^{e}
Perilla oil[j]	2.5 ± 0.8^{d}	3.9 ± 1.8^{ac}	37.4 ± 4.8^{d}	1.6 ± 2.0^{cd}	ND	ND	45.4 ± 7.0^{de}
Grape seed oil[i]	16.3 ± 2.2^{c}	5.0 ± 4.3^{ac}	8.1 ± 1.3^{e}	ND	11.2 ± 2.2^{c}	11.5 ± 2.8	52.2 ± 7.7^{d}

[a–e] The same letter in a column is not significantly different ($\alpha = 0.05$).
[f] Mean value ± standard deviation for the four black raspberry seed oils presented in Table 1.
[g] Mean value ± standard deviation of triplicates for black raspberry seed oils extracted using hot hexane.
[h] Mean value ± standard deviation of triplicates for black raspberry seed oils extracted using hot hexane and refined.
[i] Mean value ± standard deviation of three different commercial products.
[j] Mean value ± standard deviation of one commercial product and two extracted in the lab.
[k] ND: not detected.

tocopherols. Since the highly unsaturated fatty acids with 13–36% linolenic acid in the caneberry seed oils are unique compared to other vegetable oils (Oh et al., 2007), caneberry seed oils could be developed as specialty nutraceutical items. All of the pomace from caneberry wine production in Korea could be expected to permit production of about 6 metric tons of seed oils annually, derived from the determined oil yields from the caneberry seeds in a previous study (Oh et al., 2007) and the caneberry production information attributed to the personal communication from the Agricultural Technology Service Center.

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