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Fatty acids, tocopherols and proanthocyanidins in bramble seeds ☆

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Abstract

Studies were conducted on the fatty acids, tocopherols and proanthocyanidins in the seeds of 10 bramble varieties from China. The oil yields from these seeds vary from 4.81% to 15.72%. The main fatty acids in bramble seed oils are C18:2 *n*-6 (51.0–66.1%), C18:3 *n*-3 (9.70–35.6%), C18:1 *n*-9 (9.85–16.3%), and C16:0 (2.01–5.73%). The major tocopherol in all seed oils of 10 varieties was γ-tocopherol. The composition (mg/100 g) was as follows: α-tocopherol 7.65–52.6, γ-tocopherol 46.9–106, δ-tocopherol 3.1–9.50, and the active vitamin E 15.9–61.5 among the varieties. The total proanthocyanidin content varies from 6.81 to 17.6 mg/g. The main oligomers in total proanthocyanidins are dimers, and the least are trimers. The contents and composite proportions of fatty acids, tocopherols and proanthocyanidins are different according the varieties, which should be taken into account when the bramble seeds are exploited.

Keywords: Bramble seed; Fatty acids; Tocopherols; Proanthocyanidin; Composition

1. Introduction

The brambles (*Rubus species*), including raspberries, blackberries and their hybrids, belong to the Rose family (*Rosaceae*). As a new fruit in the market, brambles have recently received increasing development in China. The cultivated area has been up to 1000 hectares in China, especially in Heilongjiang, Jilin, Hebei, Jiangshu provinces and Beijing (Wang & Zhang, 2003), The income from planting brambles is lower than from traditional fruit trees, such as peach and grape in China (Xu & Chen, 2002), so more attention is focussed on the byproducts of the fruits in order to get additional benefit (The Oregon Raspberry & Blackberry Commission, 2004).

The seed oil of raspberry could be used for anti-inflammatory purposes and superior to other oils, such as virgin avocado and grape seed oil (Pourrat & Pourrat, 1973). The oil yield extracted from raspberry seed is about 10.7–18% (Oomah, Ladet, David, Liang, & Girard, 2000; Winton & Winton, 1935). As a healthy product, the fatty acids and tocopherols in the seed oil are the major components (Oomah et al., 2000), and the fatty acid compositions, in percentage, of the raspberry oil are: C16:0, 1.2–2.7; C18.0, 0.2–1.0; C18:1 *n*-9, 7.7–18.7; C18:2 *n*-6, 53.0–55.8, and C18:3 *n*-3, 29.1–35.22 (Oomah et al., 2000; Parry et al., 2005; Parry & Yu, 2004; Pourrat & Carnat, 1981). Latitudinal differences exist in the fatty acid composition of *Rubus chamaemoros* (Johansson et al., 1997). The content of tocopherols is 360 mg/100 g in the hexane extract oil of raspberry seed and the main component is the γ-isomer (Oomah et al., 2000). In cold-pressed raspberry seed oil, the total tocopherols are 88.9 mg/100 g (Parry et al., 2005).

Proanthocyanidins are a class of phenolic compounds, which are oligomers and polymers of the flavan-3-ol monomer unit. It has been reported that proanthocyanidins possess various biological activities, such as antioxidant activity (Ricardo, Darmon, Fennandez, & Mitjavila, 1991; Vinson, Dabbagh, Serry, & Jang, 1995), free radial-scavenging activity (Saint-Cricq de Gaulejac, Vivas, de Freitas, & Bourgeoi, 1999), inhibiting of platelet aggregation (Cook & Samma, 1996; Zafirov, Bredy-Dobreva, Litche, & Papasova, 1990), anti-ulcer activity (Saito,

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Hosoyama, Ariga, Kataoda, & Yamaji, 1998), and radioprotective effects (Castillo et al., 2000). Proanthocyanidins in bramble seed are considered as "a great potential" for berry growers and processors (The Oregon Raspberry & Blackberry Commission, 2004). Proanthocyanidins in the fruits of bramble are monomers, dimers and trimers; the total content in the wet fruits is about 0.13 mg/g (Ou, 2000).

Previous studies have all concentrated on raspberry seed, the oil content in blackberry seed was unknown. Additionally the content and composition of fatty acids, and tocopherols in seed oil of different varieties have not been fully clarified. Proanthocyanidins in bramble seed have not yet been reported. This paper is to compare the content and composition of fatty acids, tocopherols and proanthocyanidins, in the seed, among 10 bramble varieties and provide further guidelines for use of bramble seed.

2. Materials and methods

2.1. Plant materials and samples preparation

Berry samples of 10 bramble varieties were collected from the Experimental Orchard of the Institute of Pomology and Forestry, Beijing Academy of Agriculture and Forestry Science at Beijing. These varieties covered all the types of the bramble, which belonged to the raspberry and blackberry classes, in detail. Boyen, 986 and Tulmeen fell into the summer fruit red raspberry type, Heritage into fall fruit red raspberry, Jing Yellow into yellow raspberry, Black Pearl into black raspberry, Kiowa, Shawnee and Hull into erect or semierect blackberry, and Waldo into trailing blackberry (Crandall, 1995).

Seeds were isolated from fruits and dried in a fan-dryer (DZF-6021, Jingmi Instrument Co., Shanghai) for 4 h at 50 °C to reduce the moisture content to 5–6%. The mean weight of 100 seeds was determined. The seeds (10 g) were ground (FW100, Tast Instrument Co., Tianjin) to pass a 1 mm screen. The oil from milled samples was extracted by the method of Oomah, Mazza, and Przybylski (1996), but using petroleum ether. Briefly, the sample was stirred for 2 h at 4 °C with petroleum ether. The solvent was removed by vacuum filtration and the sample was twice further extracted. After the last filtration, the extract was pooled, and petroleum ether removed (vacuum rotary evaporation, 35 °C). Yield was determined gravimetrically to record the colour, then the oil was purged with nitrogen and stored at −20 °C prior to analysis of fatty acids and tocopherols.

The seeds were put into the open air, allowing the petroleum ether to volatilize and to dry. Proanthocyanidins were extracted as by Ou (2000). Briefly, the dry seed powder was extracted with 40 ml solvent containing acetone, water and acetic acid (79:29.5:0.5). The mixture of solvent and samples was vortexed and sonicated for 5 min in a water bath at 50 °C, and allowed to extract for 30 min, the solvent removed and the extracting twice repeated. The resulting solvent was rotary-evaporated at 50 °C under partial vacuum and the residue was diluted to 20 ml with water. The proanthocyanidin fraction was isolated using a Sephadex LH-20 column, washing with methanol/water (0:100, 20:80, 60:40, 100:0). The 100% methanol fraction was concentrated by rotary-evaporation. The concentrated material, i.e., the total proanthocyanidin, was weighed and diluted upto 20 ml in water for analysis of oligomers.

2.2. Analytical procedure

The Official Methods (The National Standard of the People of Republic of China, 1988) were used for determining fatty acid. Fatty acid Me esters were prepared by NaOMe-catalysed transesterification and analysed by GC on a SP-600 gas chromatograph (Lunan Instrument Co. Shandong) equipped with stainless steel column, filled with 12% DEGS and chromosorb PWA (60–80, HP). The injector and detector temperatures were 205 and 250 °C; the oven temp was 205 °C. The linear flow rate of the carrier gas (N_2) was 30 ml/min, H_2 30 ml/min and air 300 ml/min.

Tocopherols were analyzed with an HPLC system (Waters-2695, USA) and fluorescence detector (Waters 996). A Supelco 526 column (C18, 4.6 mm × 150 mm, 5 μm) was used with methanol/water (98/2) as mobile phase. The system was operated at a flow rate of 1 ml/min. Separations were carried out at room temperature and the wavelength was 300 nm. The sample was diluted to 2 ml with hexane, filtered and 10 μl solution were injected. The quantitative analysis was based on the external standard method. All of the procedure accorded to official methods (The National Standard of the People of Republic of China, 2004).

The proanthocyanidins were confirmed by LC/MS, which was performed on an Agilent 1100 series LC/MS Trap SL MS with TrapControl 4.2 and Bruker Daltonics Data Analysis 2.2. The HPLC apparatus was HP1100, equipped with quaternary pump, and a UV–Vis detector. The detection was at 254 nm, and the ejection was 10 μl. A gradient of solvent A (NH_4COOH, adjusting pH to 3.0 with HCOOH) and solvent B (water/methanol, 90:10, v/v): 90% A from 0 to 5 min, 90–30% A from 5 to 50 min, 30–0% A from 50 to 55 min, 0–90% A from 55 to 60 min, was applied to a reversed-phase Zorbax SB-C_{18} column (250 × 4.6 mm, 5.0 μm, Agilent Co.). Flow rate was 0.8 ml/min. Nitrogen was used as the nebulizing and drying gas. Esi conditions were as follows: nitrogen pressure, 5.00 psi; drying gas, 3.0 l/min at 325 °C; ion spray voltage, 3500 V. Mass spectra were recorded from m/z 50 to 1000.

2.3. Statistical analysis

Sampling and analyses were performed in duplicate or triplicate, and the data were presented as means ± standard deviation. Analysis of variance and least significant difference tests were conducted to identify differences

among means. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

The seeds of blackberries are heavier than those of raspberries (Table 1). The seed of yellow raspberry is the lightest, while Tulmeen, the main variety cultured in China has the heaviest seed in raspberry. In blackberry, the seed of Waldo is the lightest, only one half of Kiowa. The oil yields of raspberry seeds vary from 9.18% to 15.7% of the dry weight and that of blackberry seeds from 4.81% to 6.10% (Table 1).

The most abundant fatty acids in bramble seed oil were oleic, linoleic, and α-linolenic acids, accounting for 95.7-98.0% in raspberries, which were significantly higher ($P < 0.05$) than the 92.0–93.3% in blackberries (Table 1). The proportions of the most abundant fatty acids of the raspberry and blackberry seed oil varied among different varieties. The proportion of α-linolenicin in the seed oil of raspberry was higher ($P < 0.001$) than that in the seed oil of blackberry. This proportion was also higher than that in other fruit seed oils (Parker, Adams, Zhou, Harris, & Yu, 2003; Parry et al., 2005). The proportion of linoleic acid was lower ($P < 0.05$) in the samples of raspberry than in the samples of blackberry. The oleic acid proportion in the seed oil of raspberry was lower ($P < 0.05$) than that of blackberry. There was no stearic acid in raspberry seed oil except that of yellow raspberry (0.78%), but the stearic acid proportion in blackberry seed oil varied from 1.98% to 2.58%. The seed oil of black raspberry contained a higher proportion (4.32%) of palmitic acid, but this proportion in raspberry seed oil was lower ($P < 0.05$) than that in blackberry seed oil.

The major tocopherol was γ-tocopherol in all the varieties of bramble seed oil (Table 2), which was higher in raspberry (79.4 mg/100 g) than in blackberry (70.4 mg/100 g), but there was no significant difference ($P > 0.05$). The contents of α-tocopherol in seed oil of raspberry (33.08 mg/100 g) were about 3X that of blackberry (11.7 mg/100 g), and the content of δ-tocopherol in raspberry seed oil (7.97 mg/100 g) was also higher than that in blackberry (3.69 mg/100 g). The total tocopherol in raspberry seed oil (120 mg/100 g) was higher ($P < 0.01$) than that in blackberry (85.8 mg/100 g). Our results are lower than whose of other authors (Oomah et al., 2000), who reported that the total tocopherol was 360 mg/100 g but higher than the value reported by Parry et al. (2005). This dissimilarity should be checked by further experiment. The biologically active vitamin E content in raspberry seed oil (41.1 mg/100 g), calculated by using the formula proposed by McLaughlin and Weihrauch (1979), Zheng (1995), was also higher than

Table 1
Weight of 100 seeds, oil yield in dry seed and fatty acid compositions in bramble seed oil[a]

Classes	Varieties	100 seeds (g)[b]	Colour	Oil (wt%)[b]	Fatty acid (16.0)[c]	Fatty acid (18.0)[c]	Fatty acid (18.1)[c]	Fatty acid (18.2)[c]	Fatty acid (18.3)[c]
Raspberry	Boyen	0.14 ± 0.03z,x	Yellow	10.50% ± 0.99%z	3.30 ± 0.28z,w	ND	12.7 ± 0.47z,w	52.9 ± 0.91z	31.2 ± 0.17z
	986	0.17 ± 0.01z	Yellow	11.10% ± 1.13%z	2.45 ± 0.07z,x	ND	15.5 ± 0.56y,x	54.0 ± 0.26z,x	27.8 ± 0.41x
	Tulmeen	0.21 ± 0.04z,y,x	Yellow	9.18% ± 0.36%z	2.01 ± 0.29y,x	ND	10.8 ± 0.94z	51.6 ± 0.76z,x	35.6 ± 0.13y
	Heritage	0.14 ± 0.04z,x	Yellow	10.79% ± 1.34%z	2.63 ± 0.24z,x	ND	12.3 ± 0.64z,v	54.6 ± 3.17z,w	30.4 ± 2.20z,y,x
	Jing Yellow	0.09 ± 0.01x,u	Yellow	11.78% ± 2.58%z,y,x	2.26 ± 0.51z,x	0.78 ± 0.18z	10.7 ± 1.29z,v	51.0 ± 2.53z,x	35.1 ± 2.17z,y
	Black Pearl	0.17 ± 0.02z	Yellow	15.72% ± 2.57%z	4.32 ± 0.40w,v	ND	9.85 ± 1.03z,u	54.1 ± 2.24z,v	31.8 ± 2.88z,y,x
Blackberry	Kiowa	0.37 ± 0.04y,w	Green	4.81% ± 0.98%y,x	5.00 ± 0.13y,v	2.15 ± 0.23y	15.8 ± 0.79y	61.0 ± 1.52y,w,v	16.0 ± 0.70w
	Shawnee	0.32 ± 0.01y,w	Yellow	5.02% ± 1.69%y	5.73 ± 0.53u,v	1.98 ± 0.29y	13.6 ± 0.87y,v,u	62.5 ± 0.91y,w	15.9 ± 2.19w,v
	Hull	0.30 ± 0.01y,w	Green	6.10% ± 0.28%x	4.45 ± 0.36u,v	2.20 ± 0.37y	15.3 ± 1.39y,v	62.0 ± 3.53y,x,w,v,u	16.0 ± 5.06w,x,v
	Waldo	0.20 ± 0.04z,w,u	Yellow	5.95% ± 0.78%y,x	4.64 ± 0.23u,v	2.58 ± 0.33y	16.3 ± 1.43w,x,v	66.1 ± 0.29u	9.70 ± 1.26v

[a] Data expressed as means ± standard deviations ([b]$n = 3$,[c]$n = 2$). Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Table 2
Tocopherol contents in bramble seed oil (mg/100 g)[a]

Type	Varieties	Tocopherol (mg/100 g)			Total	Vitamin E
		α	γ	δ		
Raspberry	Boyen	39.1 ± 0.35z	80.3 ± 1.48z	9.10 ± 2.55z,y,x	128 ± 3.68z,u	47.2 ± 0.18z
	986	34.6 ± 1.48z	55.7 ± 3.75y,x	7.50 ± 2.40z,y,x	97.7 ± 4.67w,x	40.2 ± 1.09y
	Tulmeen	22.9 ± 2.26y	10.6 ± 5.23y	7.15 ± 0.49z,x	136 ± 7.00z,u	33.5 ± 2.78y,w,t
	Heritage	52.6 ± 1.63x,w	89.0 ± 6.36z	8.20 ± 0.99z	150 ± 8.98z	61.5 ± 2.27x
	Yellow					
	Jing	27.0 ± 0.28y,w	63.0 ± 8.84z,x	9.50 ± 0.99z	99.5 ± 7.57y,t,s,q	33.4 ± 0.59v,w
	Black					
	Pear	22.4 ± 2.55y,w	82.9 ± 8.41z,x	6.35 ± 1.20z,w	12 ± 4.67u,x,t	30.8 ± 1.72v,w,t
Blackberry	Kiowa	20.5 ± 3.04y,w,v	46.9 ± 8.13y,x	4.20 ± 0.85x,w	71.5 ± 4.24w,r	25.2 ± 2.24u,t
	Shawnee	7.65 ± 2.19x	101 ± 11.53z	4.05 ± 0.21y,w	112 ± 9.12z,x,t,q	17.8 ± 1.04u,r
	Hull	10.6 ± 1.34x,v	53.5 ± 5.44y	3.40 ± 0.71y,w	67.4 ± 7.50v,s,r	15.9 ± 1.90s,r
	Waldo	8.15 ± 1.06x	80.8 ± 3.82z	3.10 ± 0.42y,w	92.1 ± 3.18y,q	16.3 ± 0.67s,r

[a] Data expressed as means ± standard deviations ($n = 2$). Values followed by the same letter in the same column are nor significantly different ($P < 0.05$).

that in blackberry (18.8 mg/100 g). Heritage, the fall fruit red raspberry, hold the highest active vitamin E content (61.5 mg/100 g), the summer fruit red raspberries, including Boyen, 986 and Tulmeen, had the second highest content (40.3 mg/100 g), the yellow raspberry and black raspberry had lower contents (33.3 mg/100 g and 30.8 mg/100 g, respectively). The erect or semierect blackberry, including Kiowa, Shawnee and Hull, had a higher active vitamin E content (19.6 mg/100 g) than the trailing blackberry, Waldo, with a content of 16.3 mg/100 g.

The total proanthocyanidin contents in the seed of raspberry and blackberry vary from 6.81 to 17.6 mg/g (Table 4). The seed of fall red raspberry, Heritage, had the highest content (17.6 mg/g) of proanthocyanidins, while the seed of summer red raspberry had low content (7.31–9.28 mg/g). The proanthocyanidin content (9.04 mg/g) in seed of trailing blackberry, Waldo, was higher ($P < 0.01$) than that in erect or semierect blackberry, Kiowa, Shawnee and Hull (7.69 mg/g).

HPLC/MS is a useful method for identifying monomer, dimer and trimer of the proanthocyanidins (Ou, 2000). At the same time, the relative content of the oligomers can be calculated by comparing each ion peak area with total ion peak area (Cong & Su, 2000). According to previous data (Hammerstone, Lazarus, & Mitchell, 1999; Ou, 2000), 7 oligomers were identified from the total proanthocyanidins (Table 3). The identification of isomeric compounds will be reported in other papers. The main oligomers (in total proanthocyanidin) of bramble seed were dimers, the least were trimers (Table 4). But the proportions of oligomers were different. In seed of red and yellow raspberry, the major oligomer was dimer 1 (relative content varying from 44.0% to 51.7%); the following oligomers were dimer 2 and monomer 1; trimers were the least; the relative content was under 5%. The black raspberry, black pearl, was more different from the above two raspberries; the major dimer 2 was only 28.2%, following oligomers were monomer 1 (22.5%) and dimer 1 (19.2%). Black pearl also had the highest contents of trimers (13.4%), which were 6% lower in the other bramble. Dimer 2 was the major oligomer in seed of blackberry, except Waldo, in which dimer 1 was the major one.

The potential for production of oil as a byproduct of raspberry and blackberry seed appears to feasible from their unique chemical compositions, mostly due to the fatty acids, tocopherols and proanthocyanidins. Our research reveals that the chemical compositions are different between varieties, which may attributed to the bio-diversity in the genus *Rubus* L. So it is important to choose appropriate

Table 3
Identification of the oligomers from the total proanthocyanidin extracted from bramble seed

Oligomers	Chemical Name	Molecular weight	Molecular ion $[M-H]^-$, (fragments)	Molecular ion $[M+H]^+$, (fragments)
Monomer 1	3,3′,4′,5,7-Pentahydoxyflavan	290	289(271,245,231,205,179, 151,137)	291(273,165,153,140,123)
Monomer 2	3′,4′,5,7-Tetrahydoxyflavan-3-*O*-(3,4,5,-trihydroxybenzoyl)	442	441(289,245)	443(425,305,291,273,153)
Dimer 1	3′,4′,5,7-Tetrahydoxyflavan(4 → 8)-3,3′,4′,5,7-pentahydoxyflavan	562	561(543,435,329,289,271,245)	563(545,437,411,289,273,231)
Dimer 2	3,3′,4′,5,7-Pentahydoxyflavan(4 → 8)-3,3′,4′,5,7-pentahydoxyflavan	578	577(559,451,425,289,245)	579(561,427,289,165)
Trimer 1	3′,4′,5,7-Tetrahydoxyflavan(4 → 8)-3′,4′,5,7-tetrahydoxyflavan(4 → 8)-3,3′,4′,5,7-pentahydoxyflavan	834	833(815,707,678,543,435,289)	[a]
Trimer 2	3′,4′,5,7-Tetrahydoxyflavan(4 → 8)-3,3′,4′,5,7-pentahydoxyflavan(4 → 8)-3,3′,4′,5,7-pentahydoxyflavan	850	849(831,723,679,559,433,287,257)	[a]
Trimer 3	3,3′,4′,5,7-Pentahydoxyflavan(4 → 8)- 3,3′,4′,5, 7-pentahydoxyflavan(4 → 8)-3,3′,4′,5,7-pentahydoxyflavan	866	865(847,739,685,577,451,287,245)	[a]

[a] Not detected.

Table 4
Proanthocyanidin contents (mg/g) and relative proportions of oligomers in the total proanthocyanidin extracted from bramble seed

Classes	Varieties	Content[a]	Monomer 1 (%)	Monomer 2 (%)	Dimer 1 (%)	Dimer 2 (%)	Trimer 1 (%)	Trimer 2 (%)	Trimer 3 (%)
Raspberry	Boyen	7.31 ± 1.85z	10.5	1.02	44.0	41.2	1.19	1.41	0.70
	986	7.35 ± 1.56z	6.23	1.79	51.7	37.0	1.37	1.46	0.42
	Tulmeen	15.3 ± 1.33z	13.1	1.36	47.5	35.2	1.30	0.81	0.72
	Heritage	9.28 ± 0.49y,x	11.8	1.09	47.9	35.7	1.66	1.37	0.54
	Jing Yellow	17.6 ± 0.95z,x	5.84	1.74	45.5	42.1	1.96	2.26	0.64
	Black Pearl	11.5 ± 1.75y,x	22.5	16.8	19.2	28.2	5.25	4.29	3.83
Blackberry	Kiowa	7.48 ± 2.09z	8.43	6.46	17.6	64.1	0.77	0.69	1.92
	Shawnee	6.81 ± 0.94z,x	17.0	3.32	25.3	51.4	0.76	1.28	0.98
	Hull	9.04 ± 2.78z,x	11.5	2.40	41.7	42.0	0.53	0.75	1.14
	Waldo	8.79 ± 3.13z	10.1	7.01	50.3	27.4	2.10	1.65	1.42

[a] Data expressed as means ± standard deviations ($n = 2$). Values followed by the same letter in the same column are not significantly different($P < 0.05$).

varieties when bramble seeds are exploited, by the unique and different chemical composition of their seeds.

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