Food Chemistry 130 (2012) 785–796

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Review

Rubus fruit phenolic research: The good, the bad, and the confusing

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ARTICLE INFO

Article history:
Received 16 May 2011
Received in revised form 11 July 2011
Accepted 5 August 2011
Available online 10 August 2011

Keywords:
Flavonoids
Nonflavonoids
Rosaceae
Bramble
Caneberry

ABSTRACT

Here we attempt to clarify contemporary scientific findings of *Rubus* fruit phenolics, focusing mainly on published peer-reviewed work from the last 6 years. Our review focuses on research papers that identified phenolics of *Rubus* fruit, although other edible parts of *Rubus* plants (i.e. leaves, roots) also contain phenolics. With an increased awareness given to the potential health benefits of consuming berries high in phenolics, efforts have been directed at enhancing *Rubus* fruit quality and colour (through plant selection, harvesting, storage, etc.) for processors and consumers alike. Assessment of any progress requires knowing the state of the starting material, so effective research into *Rubus* phenolics relies upon the accurate identification of the components in *Rubus* fruit in the initial investigations. We have summarised these reports into three sections: anthocyanins, phenolic monomers other than anthocyanins, and phenolic polymers. More work is needed in identification and quantification, and further opportunities remain for deciphering and clarifying existing phenolic information for *Rubus* fruit.

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Contents

1. Introduction

Rubus fruit have long been collected and consumed worldwide (Finn, 2008; Hummer, 2010; Quideau, 2009), regardless of whether they were recognised for their possible health benefits from their natural phytochemicals or simply because they tasted good (Rao & Snyder, 2010). *Rubus* also has a pharmacological history, which was reviewed by Hummer (2010). Charred food fragments with *Rubus idaeus* L. (red raspberry) and *Rubus caesius* L. (European dewberry) are evidence of *Rubus* fruit being part of the Danish diet as early as 5600–4000 B.C.E. (Kubiak-Martens, 1999).

Today, *Rubus* fruit are considered a healthy and nutritious food, containing phenolics (references listed in Tables 1–3), vitamin C (Borges, Degeneve, Mullen, & Crozier, 2010; Mullen et al., 2002; Pantelidis, Vailakakis, Manganaris, & Diamantidis, 2007), dietary fibre (Acosta-Montoya et al., 2010; Marlett & Vollendorf, 1994; Schmeda-Hirschmann, Feresin, Tapia, Hilgert, & Theoduloz, 2005), α-tocopherol (Bushman et al., 2004; Parry et al., 2005; Xu, Zhang, Chen, & Tu, 2006), tocotrienol (Van Hoed et al., 2009), calcium (Plessi, Bertelli, & Albasini, 2007; Schmeda-Hirschmann et al., 2005), potassium (Plessi et al., 2007; Schmeda-Hirschmann et al., 2005), magnesium (Plessi et al., 2007), carotenoids (Mertz et al., 2009; Parry et al., 2005), linoleic acid (Bakowska-Barczak, Marianchuk,

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0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2011.08.022

Table 1
Anthocyanins reported in *Rubus* fruit, alphabetically by species. Red raspberry (*R. idaeus* L.) anthocyanins are well summarised in Rao and Snyder (2010). *Rubus* fruit anthocyanins are predominately cyanidin-based. Blackberries are the only *Rubus* fruit that contain acylated pigments.

Botanical name	Cultivar/common name or designation used in the references	Identification	References
R. acuminatus Sm.	Black raspberry (actually native to Asia) grown in MI, USA	Cyanidin-3-glucoside and cyanidin-3-rutinoside.	Bowen-Forbes, Zhang, and Nair (2010)
R. adenotrichos Schltdl.	Andean blackberry	Cyanidin-3-glucoside and cyanidin-3-malonylglucoside.	Mertz et al. (2007), Acosta-Montoya et al. (2010) and Gancel et al. (2010)
R. arcticus L.	Arctic raspberry	Cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside.	Maatta-Riihinen et al. (2004)
R. chamaemorus L.	Cloudberry	Cyanidin based; cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside.	Maatta-Riihinen et al. (2004) and Koponen et al. (2007)
R. coreanus Miq.	Bokbunja/Korean black raspberry	Cyanidin-3-sambubioside, cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside, pelargonidin-3-rutinoside*, delphinidin-rutinoside-unknown*, and delphinidin-glucuronide*. Samples were obtained from commercially cultivated fields of bokbunja-ju factory, so uncertain if these samples were truly *R. coreanus* based on Eu et al. (2008) findings.	Ku and Mun (2008) and Kim et al. (2011)
R. coreanus Miq.	Bokbunja/Korean black raspberry	Cyanidin-3-rutinoside (unclear if collected from the wild).	Bae, Lim, Choi, and Kang (2007)
R. coreanus Miq.	Bokbunja/Korean black raspberry	Cyanidin, pelargonidin, and delphinidin based.	Deighton et al. (2000)
R. glaucus Benth.	Andean blackberry	Cyanidin-3-glucoside and cyanidin-3-rutinoside.	Mertz et al. (2007)
R. glaucus Benth.	Andean blackberry	Cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside.	Garzon, Riedi, and Schwartz (2009) and Estupinan, Schwartz, and Garzon (2011)
R. glaucus Benth.	Andean blackberry	Cyanidin-3-glucoside, cyanidin-3-rutinoside, pelarognidin-3-rutinoside, and cyanidin-glycoside.	Vasco et al. (2009)
R. idaeus L.	Red raspberry	Cyanidin-3,5-diglucoside*, cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside*, pelargonidin-3-glucoside*, pelargonidin-3-glucosylrutinoside*, and pelargonidin-3-rutinoside*.	Mullen, Lean et al. (2002), Maatta-Riihinen et al. (2004), Kassim et al., (2009) and Remberg et al. (2010)
R. jamaicensis L.	Jamaican blackberry	Cyanidin-3-glucoside and cyanidin-3-malonylglucoside.	Bowen-Forbes et al. (2010)
R. moluccanus L.	Molucca raspberry	Cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin-3-rutinoside.	Netzel, Netzel, Tian, Schwartz, and Konczak (2006)
R. occidentalis L.	Black raspberry	Cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside, pelargonidin-3-rutinoside*, and peonidin-3-rutinoside*.	Harborne and Hall (1964), Hong and Wrolstad (1990), Stoner et al. (2005), Tian et al. (2006a,b), Tulio et al. (2008), Dossett et al. (2008) and Dossett et al. (2010), Ling et al. (2009) and Wyzgoski et al. (2010)
R. occidentalis L.	Black raspberry mutant	Cyanidin-3-sambubioside, cyanidin-3-glucoside, and pelargonidin-3-glucoside.	Dossett et al. (2011)
R. odoratus L.	Flowering raspberry	Cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside.	Harborne and Hall (1964)
R. parviflorus L.	Thimbleberry	Cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin-3-glucoside.	Harborne and Hall (1964) and Maatta-Riihinen et al. (2004)
R. pinnatus Willd.	South African blackberry	Cyanidin-3-glucoside and cyanidin-3-rutinoside.	Byamukama et al. (2005)
R. racemosus Roxb.	Black raspberry (actually native to Asia) grown in Jamaica	Cyanidin-3-glucoside and cyanidin-3-rutinoside.	Bowen-Forbes et al. (2010)
R. rigidus Sm.	South African trailing blackberry	Cyanidin-3-glucoside and cyanidin-3-rutinoside.	Byamukama et al. (2005)
R. rosifolius Sm.	Red raspberry (actually native to Asia and Pacific Islands) grown in Jamaica	Cyanidin-3-glucoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside.	Bowen-Forbes et al. (2010)
Rubus spp. not specified; *R. laciniatus* Willd.; *R. fruticosus* aggr.	Blackberry	Cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xyloside*, cyanidin-3-malonylglucoside, and cyanidin-3-	Stintzing et al. (2002) and Fan-Chiang and Wrolstad (2005)

Table 1 (*continued*)

Botanical name	Cultivar/common name or designation used in the references	Identification	References
		dioxalylglucoside*.	
Parentage unknown; generally considered a complex hybrid containing *R. ursinus* Cham. & Schtdl., *R. idaeus* L., and *R. baileyanus* Britton.	Blackberry raspberry hybrid 'Boysenberry'	Cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside.	Fan-Chiang and Wrolstad (2005), Ghosh, McGhie, Zhang, Adaim, and Skinner (2006) and McGhie et al. (2006)
Parentage unknown; generally considered as *R. ursinus* Cham. & Schltdl. × *R. idaeus* L.	Blackberry raspberry hybrid 'Loganberry'	Cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside. Scalzo et al. (2008) reports additional cyanidin-3-xylosylrutinoside and cyanidin-3-sambubioside.	Fan-Chiang and Wrolstad (2005) and Scalzo et al. (2008)
Has multiple species in its pedigree including *R. ursinus* Cham. & Schltdl., *R. idaeus* L. and *R. armeniacus* Focke	Blackberry cultivar Marion	Cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xyloside, cyanidin-3-malonylglucoside, and cyanidin-3-dioxalylglucoside.	Siriwoharn et al. (2004) and Fan-Chiang and Wrolstad (2005)
Complex hybrid that includes *R. ursinus* Cham. & Schltdl. × *R. idaeus* selections	Blackberry raspberry hybrid 'Tayberry'	Cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, and cyanidin-3-sambubioside.	Winterhalter (2007)

* Not detected in all samples.

& Kolodziejczyk, 2007; Bushman et al., 2004; Kim et al., 2011; Parry et al., 2005; Van Hoed et al., 2009), and linolenic acid (Bakowska-Barczak et al., 2007; Bushman et al., 2004; Parry et al., 2005; Van Hoed et al., 2009).

The worldwide popularity of *Rubus* fruit has increased in part due to the repeated published accounts of highly coloured berries/fruit and their potential health benefits (Mullen, Stewart et al., 2002; Yokozawa et al., 1998; Bakkalbsai, Mentes, & Artik, 2009; Beekwilder et al., 2005; Nohynek et al., 2006; Puupponen-Pimia et al., 2005; Rao & Snyder, 2010; Ross, McDougall, & Stewart, 2007; Seeram, 2008). While more work is needed to better elucidate the mechanisms between phenolics and their promising rewards, recent well-written assessments of phenolics, their metabolites and bioavailability have started to become available (Bakkalbsai et al., 2009; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Okuda et al., 2005; Selma, Espin, & Tomas-Barberan, 2009). In order for phenolics in *Rubus* fruit to be monitored in food processing, food stability, animal and human metabolomic research, or any other investigation, a complete and accurate identification of the initial material is required. It is impossible to monitor or verify the metabolites if the phenolics initially present are misidentified.

A large body of research has been conducted on the composition of phenolics in red raspberry (*R. idaeus*), including a recent summary by Rao and Snyder (2010). Only red raspberry phenolic references cited in the body of this review in support of our assessment have been summarised in the tables. This review paper will focus on other *Rubus* fruit, including blackberry (*Rubus fruticosus* aggr., *Rubus glaucus* Benth., and *Rubus adenotrichos* Schltdl. and the interspecific hybrids between blackberry and red raspberry such as 'Boysenberry' and 'Loganberry'), black raspberry (*Rubus occidentalis* L.), arctic raspberry (*Rubus arcticus* L.), cloudberry (*Rubus chamaemorus* L.), thimbleberry (*Rubus parviflorus* Nutt.), etc. While other parts of many *Rubus* plants are edible and contain phenolics (Beekwilder et al., 2005; Dall'Acqua et al., 2008; Foo & Porter, 1981; Gudej & Tomczyk, 2004; Hukkanen, Kostamo, Karenlampi, & Kokko, 2008; Okuda et al., 1992; Tanaka et al., 1993), such as the leaves, which have traditionally been used in remedies for problems such as stomach pain (Dall'Acqua et al., 2008; Hummer, 2010), this review focuses on the fruit.

Our objective was to clarify and compile a comprehensive summary of the phenolic identifications that have been performed on *Rubus* fruit. If independent examinations identified similar compounds from comparable samples, their results have been combined in the following data, if differing phenolic identifications were made then they have been listed separately for each of the following three tables: anthocyanins (Table 1), phenolic monomers other than anthocyanins (Table 2), and phenolic polymers (Table 3). A few instances of misidentification generating misunderstanding in the phenolic literature have also been summarised in this paper.

2. Phenolics reported in *Rubus* fruit

Even before the fruit enters the laboratory for phytochemical extraction and analysis their phenolic profiles and concentrations will vary because of genetic (i.e. genus, species, cultivar/genotype) and environmental (i.e. fruit maturity, plant age, growing season, field location) factors (Acosta-Montoya et al., 2010; Beekwilder et al., 2005; Dossett, Lee, & Finn, 2008, 2010, 2011; Fan-Chiang & Wrolstad, 2005; Gasperotti, Masuero, Vrhovsek, Guella, & Mattivi, 2010; Johnson, Bomser, Scheerens, & Giusti, 2011; Lee & Finn, 2007; Maatta-Riihinen, Kamal-Eldin, & Torronen, 2004; Ozgen et al., 2008; Plessi et al., 2007; Sariburun, Sahin, Demir, Turkben, & Uylaser, 2010; Siriwoharn, Wrolstad, Finn, & Pereira, 2004; Vrhovsek, Giongo, Mattivi, & Viola, 2008). Solar radiation, temperature, virus status, and other biotic and abiotic stresses also affect phenolic content (Lee & Martin, 2009; Remberg, Sonsteby, Aaby, & Heide, 2010; Tarara, Lee, Spayd, & Scagel, 2008). Anthocyanins from black raspberries ($n = 190$ genotypes), from a single location collected from two field seasons, analysed for a selective breeding project ranged from 245 to 541 mg/100 ml (Dossett et al., 2010). This variability illustrated genotypic differences in anthocyanin levels despite uniform research field conditions.

There is no scientific standard for storing, preparing, extracting, purifying, analysing, or reporting individual phenolics from any berry/fruit, which leads to diverse reports and confusion when comparing results in the literature. Phenolics can be extracted from fresh or preserved fruit that are usually frozen or freeze-dried (Dossett et al., 2008, 2010; Fan-Chiang & Wrolstad, 2005; Gancel, Feneuil, Acosta, Perez, & Vaillant, 2010; Mullen, Stewart et al., 2002; Turkben, Saiburun, Demir, & Uylaser, 2010). The extraction methods that have been used range from analysing whole-fruit extracts to analysis of fruit juice, further complicating the ability to draw comparisons because concentrations may be expressed as

Table 2
Phenolic monomers other than anthocyanin reported in *Rubus* fruit, alphabetically by species. Red raspberry (*R. idaeus* L.) phenolic monomers are well summarised in Rao and Snyder (2010). Hydroxycinnamic acids, hydroxybenzoic acids, flavanols, and flavonol-glycosides are the classes of compounds reported in *Rubus* fruit.

Botanical name	Cultivar/common name or designation used in the references	Identification	References
R. adenotrichos Schltdl.	Andean blackberry	Quercetin-glycosides, ellagic acids and derivatives.	Acosta-Montoya et al. (2010) and Gancel et al. (2010)
R. adenotrichos Schltdl.	Andean blackberry	Gallic acid, gallic acid derivatives, caffeic acid, coumaric acid, ferulic acid derivatives, epicatechin, ellagic acid derivatives, quercetin-glycosides, and kaempferol-glycosides.	Mertz et al. (2007)
R. arcticus L.	Arctic raspberry	Caffeoylglucoside, *p*-coumaroyl sugar esters, ferulic acid esters, galloyl esters, ellagic acid derivative, ellagic acid, catechin, epicatechin, quercetin-3-glucorone-deoxyhexoside, quercetin-3-glucuronide, kaempferol-3-glucuronide, and isorhamnetin-3-glucuronide.	Maatta-Riihinen et al. (2004)
R. chamaemorus L.	Cloudberry	*p*-Coumaric acid, caffeic acid, ferulic acid, gallic acid, ellagic acid derivative, ellagic acid, catechin, epicatechin, and quercetin-3-glucuronide.	Maatta-Riihinen et al. (2004)
R. chamaemorus L.	Cloudberry	Ellagic acid, hydroxybenzoic acids, hydroxycinnamic acids, and flavonols.	Laine et al. (2008)
R. chamaemorus L.	Cloudberry	Caffeic acid, ferulic acid, sinapic acid, vanillic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, cinnamic acid, and gallic acid.	Mattila et al. (2006)
R. chamaemorus L.	Cloudberry	Hydroxycinnamic acids, catechin, and epicatechin.	Puupponen-Pimia et al. (2005) and Nohynek et al. (2006)
R. coreanus Miq.	Bokbunja/Korean black raspberry	Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, salicylic acid, caffeic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, and cinnamic acid.	Ju et al. (2009)
R. coreanus Miq.	Bokbunja/Korean black raspberry	Ferulic acid, epicatechin, protocatechuic acid, gallic acid, vanillic acid, and cinnamic acid.	Kim et al. (2011)
R. fruticosus aggr.	Blackberry	Ellagic acid.	Hager et al. (2008) and Hager et al. (2010)
R. fruticosus aggr.	Blackberry	Ellagic acid, catechin, epicatechin, rutin (quercetin-3-rutinoside), and quercetin.	Jakobek et al. (2009)
R. fruticosus aggr.	Blackberry	Ellagic acid.	Gasperotti et al. (2010)
R. fruticosus aggr.	Blackberry	Ellagic acid, ferulic acid, caffeic acid, *p*-coumaric acid, and quercetin. Analysis conducted after hydrolysis.	Turkben et al. (2010)
R. glaucus Benth.	Andean blackberry	Gallic acid, gallic acid derivatives; caffeic acid, coumaric acid and ferulic acid derivatives; epicatechin, ellagic acid derivatives, and quercetin-glycosides.	Mertz et al. (2007)
R. glaucus Benth.	Andean blackberry	Gallic acid, 2 ellagic acid glycosides, ellagic acid, ellagic acid derivative, *p*-coumaroyl sugar ester, flavanol derivative, epicatechin, 2 quercetin-glycosides, quercetin-3-glucuronide, quercetin-3-arabinoside, and kaempferol-3-glucuronide.	Vasco et al. (2009)
R. idaeus L.	Red raspberry	Ellagic acid.	Gasperotti et al. (2010)
R. idaeus L.	Red raspberry	Ellagic acid pentoside 1, ellagic acid pentoside 2, ellagic acid, methyl ellagic acid pentoside, and ellagic acid 4-acetylxyloside.	Remberg et al. (2010)
R. idaeus L.	Red raspberry	*p*-Coumaric acid, ferulic acid, ellagic acid, and quercetin.	Jakobek et al. (2009)
R. idaeus L.	Red raspberry	Quercetin-3,4-diglucoside, ellagic acid pentose conjugates, quercetin-galactosylrhamnoside, quercetin-3-rutinoside, quercetin-3-glucoside, quercetin-glucuronide, methyl ellagic acid-pentose conjugates, ellagic acid-4-acetylxyloside, and ellagic acid-4-acetylarabinoside.	Mullen, McGinn et al. (2002) and Mullen et al. (2003)
R. idaeus L.	Red raspberry	Ellagic acid, ferulic acid, caffeic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and quercetin. Analysis conducted after hydrolysis.	Turkben et al. (2010)
R. occidentalis L.	Black raspberry	Ellagic acid, ferulic acid, *p*-coumaric acid, and quercetin.	Stoner (2009)
Unreported	Blackberry	Gallocatechin, catechin, epigallocatechin, and epicatechin.	De Pascual-Teresa, Santos-Buelga, and Rivas-Gonzalo (2000)
Parentage unknown; generally considered a complex hybrid containing *R. ursinus* Cham. & Schtdl., *R. idaeus* L., and *R. baileyanus* Britton.	Blackberry raspberry hybrid 'Boysenberry'	Catechin, epicatechin, ellagic acid, quercetin-arabinoside, quercetin-glucuronide, and kaempferol-*p*-coumaroyl-glucoside. These identification was conducted on juice and seeds obtained from a processor.	Furuuchi et al. (2011)
Parentage unknown; generally considered as *R. ursinus* Cham. & Schltdl. × *R. idaeus* L.	Blackberry raspberry hybrid 'Loganberry'	Ellagic acid; identified from loganberry wine precipitates. These wines were made with no contact with oak, since oak is another source of ellagic acid.	Singleton et al. (1966)
Has multiple species in its pedigree including *R. ursinus* Cham. & Schltdl., *R. idaeus* L. and *R. armeniacus* Focke.	Blackberry cultivar Marion	Gallic acid, flavonol-glycoside unknowns, quercetin-glycoside, kaempferol-glycosides, and ellagic acid.	Siriwoharn et al. (2004) and Siriwoharn and Wrolstad (2004)

an amount/fresh weight (Fan-Chiang & Wrolstad, 2005; Hakkinen, Karenlampi, Mykkanen, Heinonen, & Torronen, 2000; Koponen, Happonen, Mattila, & Torronen, 2007; Hager et al., 2010; Acosta-Montoya et al., 2010; and many other references herein), amount/volume of juice (Dossett et al., 2008, 2010, 2011; Furuuchi, Yokoyama, Watanabe, & Hirayama, 2011; Johnson et al., 2011), or

Table 3
Phenolic polymers found in *Rubus* fruit, alphabetically by species. Red raspberry (*R. idaeus*) phenolic polymers are again, well summarised in Rao and Snyder (2010). Identification of *Rubus* polymers is an area requiring further investigated. Polymer analyses are complicated by a lack of commercially available standards.

Botanical name	Cultivar/common name or designation used in the references	Identification	References
R. adenotrichos Schltdl.	Andean blackberry	Lambertianin C and sanguiin H-6.	Mertz et al. (2007), Acosta-Montoya et al. (2010) and Gancel et al. (2010)
R. arcticus L.	Arctic raspberry	2 ellagitannins.	Maatta-Riihinen et al. (2004)
R. chamaemorus L.	Cloudberry	Proanthocyanidin dimer B2 and 2 ellagitannins.	Maatta-Riihinen et al. (2004) and Koponen et al. (2007)
R. chamaemorus L.	Cloudberry	Proanthocyanidin dimer B3, B1, and B4, trimer B, and ellagitannins.	Puupponen-Pimia et al. (2005) and Nohynek et al. (2006)
R. chamaemorus L.	Cloudberry	Ellagitannins and proanthocyanidins.	Laine et al. (2008)
R. fruticosus aggr.	Blackberry	16 ellagitannins (12 tentatively identified, lambertianin C, and sanguiin H-6) and ellagic acid conjugates (2). Lambertianin C and sanguiin H-6 are the main ellagitannins.	Gasperotti et al. (2010)
R. fruticosus aggr.	Blackberry	Ellagitannins (hydrolysis products- methylgallate, ellagic acid derivative, ellagic acid, and methylsanguisorboate).	Vrhovsek et al. (2006, 2008)
R. fruticosus aggr.	Blackberry	2 pedunculagin isomers[*], 2 castalagin/vescalagin isomers[*], galloyl-HHDP (hexahydroxydiphenic) glucose isomer, 2 lambertianin C isomers, 2 sanguiin H-6/lambertianin A, lambertianin D isomer, galloyl-bis-HHDP glucose isomer, 2 unknowns.	Hager et al. (2008) and Hager et al. (2010)
R. glaucus Benth.	Andean blackberry	Lambertianin C and sanguiin H-6.	Mertz et al. (2007)
R. glaucus Benth.	Andean blackberry	2 ellagitannins.	Vasco et al. (2009)
R. idaeus L.	Red raspberry	12 ellagitannins (10 tentatively identified, lambertianin C, and sanguiin H-6) and ellagic acid conjugates (3).	Gasperotti et al. (2010)
R. idaeus L.	Red raspberry	Lambertianin C and sanguiin H-6.	Remberg et al. (2010)
R. idaeus L.	Red raspberry	Sanguiin H-10, lambertianin C, sanguiin H-6, and nobotanin A-/malabathrin B-like.	Mullen, Stewart et al. (2002), Mullen, McGinn et al. (2002) and Mullen et al. (2003)
R. idaeus L.	Red raspberry	Lambertianin C, sanguiin H-6, and 2 proanthocyanidins.	Beekwilder et al. (2005)
Unreported	Blackberry	Proanthocyanidin B1, proanthocyanidin dimer B3, and epicatechin-(4,8)-epicatechin-(4,8)-catechin.	De Pascual-Teresa et al. (2000)
Parentage unknown; generally considered a complex hybrid containing *R. ursinus* Cham. & Schtdl., *R. idaeus* L., and *R. baileyanus* Britton.	Blackberry raspberry hybrid ‘Boysenberry’	Galloyl-sanguiin H-6 (possibly lambertianin C artifact), sanguiin H-6, and sanguiin H-2.	Kool et al. (2010)
Parentage unknown; generally considered a complex hybrid containing *R. ursinus* Cham. & Schtdl., *R. idaeus* L., and *R. baileyanus* Britton.	Blackberry raspberry hybrid ‘Boysenberry’	Procyanidin B3, B4, C2, and trimer, two propelargonidin dimers and six propelargonidin trimers, two peduculagin or its isomers, two sanguiin H-10 or its isomers, two bis HHDP galloylglucosides, sanguiin H-6, lambertianin A, sanguiin H-2 or its isomer, lambertianin C. These identification was conducted on juice and seeds obtained from a processor.	Furuuchi et al. (2011)
Has multiple species in its pedigree including *R. ursinus* Cham. & Schltdl., *R. idaeus* L. and *R. armeniacus* Focke.	Blackberry cultivar Marion	Ellagitannins and proanthocyanidins.	Siriwoharn et al. (2004) and Siriwoharn and Wrolstad (2004)

[*] As mentioned in this review article, these compounds warrant confirmation by other research groups.

an amount per fruit/berry (Cohen, Tarara, & Kennedy, 2008). In addition, phenolic quantification from cryogenically milled fruit extracts (Fan-Chiang & Wrolstad, 2005; Lee & Finn, 2007; Lee & Rennaker, 2011; Lee & Schreiner, 2010) differed from quantification from simply pureed/blended fruit (Dossett et al., 2008, 2010, 2011; Lee & Rennaker, 2011; Lee & Schreiner, 2010), where the seeds were largely left intact; seeds of many *Rubus* species are considered to be high in phenolics (Bushman et al., 2004; Hager, Howard, Liyanage, Lay, & Prior, 2008).

Solvents are then used to chemically extract the phenolics from the samples. As a compound’s solubility is solvent dependant, accurate analysis is dependant upon the solvents utilised (Lee & Rennaker, 2011; Turkben et al., 2010). Due to the time and effort our laboratory invests in exhaustive chemical extractions (Lee & Finn, 2007; Lee & Rennaker, 2011), we have tailored fruit extraction procedures to accommodate a large number ($n > 1100$) of extraction samples for some projects (Dossett et al., 2008, 2010, 2011). Phenolic extractions can comprise of many solvents and conditions. Possible solvents include water, acetone, methanol, ethanol, and ethyl acetate, while solvent composition is further altered by the addition of various acids. Conditions are adjusted further by varying solvent ratios (acid to solvent, solvent to solvent, sample to solvent), extraction temperatures, and extraction duration (Kim & Verpoorte, 2010).

Clean-up aids also have some influence on a sample’s final extract. After samples have undergone primary extraction, these crude extracts may then be purified by solid phase extraction using materials like Sep-pak C18 (reversed phase sorbent; silica based bonded phase; Waters Corp., Milford, MA, USA), polyamide SC6 (size exclusion; nylon; Macherey–Nagel GmbH and Co., Duren, Germany), Dowex 50W-X8 (ion exchange; styrene-DVB gel; Dow Chemical Company, Midland, MI, USA), Amberlite XAD-7HP (ion exchange; macroreticular aliphatic cross-linked polymer absorbent; Rohm and Haas Company, Philadelphia, PA, USA), Sephadex LH-20 (gel filtration; hydroxypropylated cross-linked dextran beads; GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), Diaion HP-20 (ion exchange; styrene–divinylbenzene; Mitsubishi Chemical Corp., Tokyo, Japan), and many others (Byamukama, Kiremire, Andersen, & Sterigen, 2005; Furuuchi et al., 2011; Gasperotti et al., 2010; Kellogg et al., 2010; Kool, Comeskey, Cooney, & McGhie, 2010; Mertz, Cheynier, Gunata, & Brat, 2007; Mullen et al., 2002; Ross et al., 2007; Salminen & Karonen, 2011 and supplementation section; Siriwoharn & Wrolstad, 2004). Sample preparation methods, solid phase extraction, and their influence on plant metabolite analysis were well reviewed previously (Kim & Verpoorte, 2010; Poole, 2003).

Poor preparation techniques can cause partial hydrolysis and underestimate ellagitannin levels in blackberries (Siriwoharn & Wrolstad, 2004; Siriwoharn et al., 2004). This may explain why they observed vast concentration variations among their extracts of aliquots from a uniform sample (as pointed out by Siriwoharn & Wrolstad, 2004), and why their values (8–27 mg/100 g ellagitannins fw from $n = 11$; average 19 mg/100 g) were so much lower from what others had previously measured (additional examples listed below). A recent example of measured concentrations comes from Gasperotti et al. (2010), who reported ellagitannin levels in their five blackberry cultivars (Apache, Chesapeake, Loch Ness, Thornfree, and Triple Crown) to range from 85 to 130 mg/100 g fw (average 108 mg/100 g).

Some researchers take further preparation steps with enzyme or acid hydrolysis (Maatta-Riihinen et al., 2004; Siriwoharn & Wrolstad, 2004; Bushman et al., 2004; Mattila, Hellstrom, & Torronen, 2006; Mertz et al., 2007; Turkben et al., 2010) to examine ellagitannin by identifying its easier to detect component compounds. What is eventually detected by HPLC separation and diode array and/or mass spectrometer (MS) detection is a complex composite that can be enhanced or hindered by choices introduced at each of the various sample preparation steps.

The difficulty of analysing polymers (Gasperotti et al., 2010) has only added to the confusion of contradictory articles and identifications. Any report of interest should be carefully scrutinised if a researcher intends to emulate the method for their own analysis, since extraction procedures (Lee & Rennaker, 2011; Lee & Schreiner, 2010), standards used to report phenolics (Dossett et al., 2008; Lee & Rennaker, 2009), methods for measuring phenolics (Dossett et al., 2008, 2010; Lee & Rennaker, 2009), etc. can alter the qualitative and quantitative results.

Again, in our tables summarising phenolic composition, references corroborating phenolic identifications for an analogous fruit were combined. If the phenolic identifications were vastly different for a same common name, they were separated into individual rows within each table.

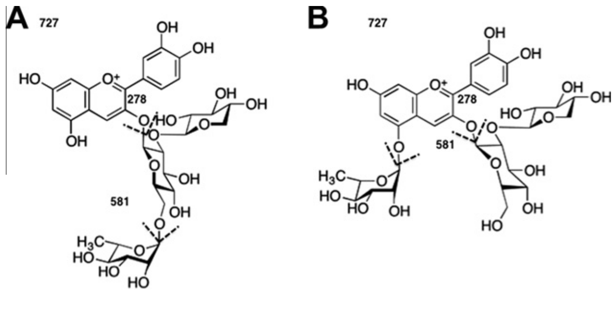
2.1. Anthocyanins

Anthocyanins identified in *Rubus* fruit are summarised in Table 1. Anthocyanins from *Rubus* fruit are unique in that they are predominately cyanidin based in the non-acylated form (Table 1; all references therein). Acylated pigments are occasionally found in *Rubus* fruit at low concentrations (Fan-Chiang & Wrolstad, 2005; Stintzing, Stintzing, Carle, & Wrolstad, 2002). So far, most blackberries (cultivars Black Douglas, Chester, Evergreen, Marion, etc.) have been reported to contain acylated pigments like cyanidin-3-malonylglucoside and cyanidin-3-dioxalylglucoside (Fan-Chiang & Wrolstad, 2005; Stintzing et al., 2002). Minor levels of pelargonidin and peonidin based anthocyanins have been found in *Rubus* fruit (Beekwilder et al., 2005; Deighton, Brennan, Finn, & Davis, 2000; Dossett et al., 2008, 2010, 2011; Harborne & Hall, 1964; Mullen, Lean, & Crozier, 2002; Mullen, Stewart et al., 2002; Tian, Giusti, Stoner, & Schwartz, 2005). Delphinidin based anthocyanins have been reported in five accessions of *Rubus coreanus* Miq., two accessions of *Rubus ursinus* Cham. & Schltdl., two accessions of *Rubus innominatus* S. Moore, *Rubus ulmifolius* Schott, *Rubus parvifolius* L., *Rubus caucasicus* Focke, *Rubus niveus* Thunb., and *R. idaeus* (Deighton et al., 2000). Unfortunately these identifications were based on anthocyanidins instead of individual anthocyanins, and additional work will be required to confirm the findings. Deighton et al. (2000) also reported peonidin-based anthocyanins in two accessions of *R. ursinus*, two accessions of *R. innominatus*, *R. ulmifolius*, *R. parvifolius*, *R. caucasicus*, and *R. niveus*. Malvidin based anthocyanins, the other naturally occurring anthocyanidin, have only been reported once in *Rubus* fruit: by Kellogg et al. (2010), but this identification work needs to be confirmed. Kellogg et al. (2010) reported finding cyanidin-3-arabinoside and malvidin-3-galactoside in cloudberries collected from the wild in Alaska. As these identifications have not yet been substantiated by any other published analysis of cloudberries, we are hesitant to accept their findings. Cuevas-Rodriguez et al. (2010) reported minor levels of cyanidin-3-arabinoside in Mexican blackberries (*Rubus* spp.), but this report needs to be confirmed as well.

The HPLC elution order of anthocyanins in *Rubus* fruit and tools for their identification have been well established (Giusti, Rodriguez-Sanoa, Griffin, & Wrolstad, 1999; Hong & Wrolstad, 1990). *Rubus* fruit anthocyanins have been clearly identified by others as well (Dossett et al., 2008; Hong & Wrolstad, 1990; Rao & Snyder, 2010). *Rubus* fruit (blackberry, red raspberry, 'Boysenberry' [a blackberry × raspberry hybrid], and black raspberry) anthocyanins have also been misidentified; e.g. Wada and Ou (2002) and since pointed out by Fan-Chiang and Wrolstad (2005) and McGhie, Rowan, and Edwards (2006). Wada and Ou (2002) made the mistake of identifying anthocyanins chiefly from HPLC–MS data, which as some anthocyanins share the same mother and fragmentation ions (Dossett et al., 2008; Giusti, Rodriguez-Sanoa, Griffin et al., 1999; Lee & Finn, 2007), can lead to misidentification. In addition to peak *m/z*, the HPLC anthocyanin peak-area information such as retention time, UV–VIS spectra, and co-chromatography with an authentic standard are required to properly identify these compounds (Dossett et al., 2008, 2011; Giusti, Rodriguez-Sanoa, Griffin et al., 1999). Unfortunately, despite this error, a recent literature database search (Scopus search accessed on 7/11/2011) shows that Wada and Ou (2002) have been cited over 110 times.

The main anthocyanin in black raspberry (cyanidin-3-xylosylrutinoside) has been correctly identified and published in the literature before 2004 (Harborne & Hall, 1964; Hong & Wrolstad, 1990), incorrectly (Prior et al., 2009; Seeram et al., 2006; Wu, Pittman, & Prior, 2006; Wu & Prior, 2005), and then corrected and reconfirmed again by several groups (Dossett et al., 2008, 2010, 2011; Ling et al., 2009; Stoner et al., 2005; Tian, Giusti, Stoner, & Schwartz, 2006a,b; Tian et al., 2005; Tulio et al., 2008; Wyzgoski et al., 2010). The cyanidin-3-xylosylrutinoside peak has been incorrectly identified as cyanidin-3-sambubioside-5-rhamnoside by a few researchers (Prior et al., 2009; Seeram et al., 2006; Wu & Prior, 2005; Wu et al., 2006), though as reported by Dossett et al. (2008) both share the same molecular (*m/z* 727) and fragmentation (*m/z* 581 and 287) ions. In Fig. 1, the fragmentation points of these two anthocyanins are illustrated. Anthocyanins with 3-glycosides and 3,5-diglycosides each have unique UV–VIS spectra (Andersen, 1985; Giusti, Rodriguez-Sanoa, & Wrolstad, 1999; Hong & Wrolstad, 1990; Dossett et al., 2008). A comparison of the UV–VIS spectra from the two anthocyanins (3-glycosides versus 3,5-diglycosides) can be found in Dossett et al. (2008); and they are known to have different absorptions (Andersen, 1985; Giusti, Rodriguez-Sanoa, Wrolstad et al., 1999; Hong & Wrolstad, 1990; Dossett et al., 2008). Anthocyanins with 3-glycosides absorb stronger around 400–460 nm compared to 3,5-diglycosides. Some researchers report this as a ratio of $\text{Absorbance}_{400}/\text{Absorbance}_{\max}$, which gives a larger value for 3-glycosides compared to 3,5-glycosides containing anthocyanins. The correct peak identification of cyanidin-3-xylosylrutinoside has also been re-confirmed by Nuclear Magnetic Resonance (NMR) spectroscopy (Tulio et al., 2008).

Adding to the confusion, the misidentified black raspberry anthocyanins were unwittingly then used for *in vitro* and *in vivo* studies to better understand their pharmacokinetic mechanisms (Prior et al., 2009; Seeram et al., 2006; Wu et al., 2006). However, without correct identifications, those black raspberry anthocyanin consumption-tracking findings become questionable. Researchers



cyanidin-3-xylosylrutinoside cyanidin-3-sambubioside-5-rhamnoside

Fig. 1. Black raspberry (*Rubus occidentalis* L.) anthocyanin: cyanidin-3-xylosylrutinoside. Correctly identified as (A) cyanidin-3-xylosylrutinoside (Dossett et al., 2008, 2010; Hong & Wrolstad, 1990; Tian et al., 2005; Tian et al., 2006a,b; Tulio et al., 2008) and misidentified as (B) cyanidin-3-sambubioside-5-rhamnoside (Prior et al., 2009; Seeram et al., 2006; Wu & Prior, 2005; Wu et al., 2006). Both anthocyanins have an identical molecular ion (*m/z* of 727) and fragmented ions (*m/z* 581 and 287), but each has a distinct UV–VIS spectrum, as discussed in this review. Dotted lines indicate fragmentation points yielding the same fragmented ions of *m/z* 581 and 287.

conducting animal and human studies on black raspberry or other anthocyanins, who unwittingly trust inaccurate work, could be tracing the pathways of unintended compounds. The correct black raspberry anthocyanin identifications can be found in references listed in Table 1.

Another example of plant misidentification potentially leading to anthocyanin confusion was clarified by Eu et al. (2008), who reported that cultivated Bokbunja fruit (bokbunja-ddal-gi), widely regarded as *R. coreanus* was actually *R. occidentalis*, based on comparisons of random amplified polymorphic DNA markers, as well as flower and leaf morphology. As a result, reports based on the analysis of commercially obtained and presumed *R. coreanus* fruit are suspect, compared to data from verified *R. coreanus*. All phenolic chemists should take care when planning future *R. coreanus* research utilising commercial sources to ensure that the source material is truly from *R. coreanus.*

Confusion surrounding *Rubus* species taxonomy and systematics has also added a hodgepodge of additional anthocyanin (and other phenolics) identifications to the literature. Scalzo, Currie, Stephens, McGhie, and Alspach (2008) analysed black raspberry plants ($n = 124$) from Motueka, New Zealand, finding cyanidin-3-sophoroside (main anthocyanin found in red raspberry; Rao & Snyder, 2010) in the anthocyanin profile in 95 out of the 124 black raspberry genotypes. As pointed out by Dossett et al. (2010), those 95 were not pure black raspberry, but had instead been bred with red raspberry, primarily to introduce genes for thornlessness (botanically known as spinelessness). Interspecific hybridisation has played an important role in blackberry and raspberry breeding (Clark, Stafne, Hall, & Finn, 2007; Hall, Hummer, Jamieson, Jennings, & Weber, 2009) with the result that many cultivars may have as many as three or four different *Rubus* species in their ancestry. This can only lead to confusion and underscores the need for studies examining the anthocyanin composition of a variety of wild and cultivated *Rubus* species to better understand anthocyanins present in this germplasm and the potential effects of using these species in future breeding.

In Dossett et al. (2011), we reported on a newly discovered wild black raspberry with a unique anthocyanin profile. These plants were wild collected and then grown in a research plot as part of investigations on genetic diversity available for breeding improved black raspberry. These black raspberry mutants had a unique anthocyanin profile where the fruit lacked anthocyanins containing rutinosides. While their distinctive profile provides an opportunity to study the genetic control over that portion of the anthocyanin biosynthetic pathway, it also shows that opportunities remain for discovering new anthocyanin profiles in even widely studied *Rubus* fruit.

2.2. Phenolic monomers other than anthocyanins

For conciseness within this review, phenolic monomers other than anthocyanins will be referred to as phenolic monomers, and are summarised in Table 2. Phenolic acids (free and conjugated forms of hydroxycinnamic and hydroxybenzoic acids), flavanol monomers (catechin and epicatechin), and flavonol-glycosides (quercetin- and kaempferol-glycosides) make up the three categories of phenolic monomers that have been reported in *Rubus* fruit (Table 2; all references therein). These compounds are typically lower in quantity than ellagitannins or anthocyanins, when all three groups are analysed together in *Rubus* fruit (Maatta-Riihinen et al., 2004; Mertz et al., 2007; Acosta-Montoya et al., 2010; Borges et al., 2010; Furuuchi et al., 2011; Jakobek, Seruga, Seruga, Novak, & Medvidovic-Kosanovic, 2009; Vasco, Riihinen, Ruales, & Kemal-Eldin, 2009). For example, the phenolic composition of arctic raspberries (*R. arcticus*) was 9% phenolic monomers < 24% anthocyanins < 67% ellagitannins (Maatta-Riihinen et al., 2004). The phenolic composition of *R. glaucus* fruit was 5% phenolic monomers < 24% anthocyanins < 71% ellagitannins and *R. adenotrichos* fruit had 7% phenolic monomers < 39% anthocyanins < 55% ellagitannins (Mertz et al., 2007). On a taxonomy side note, as *R. adenotrichos* is frequently misspelled as *Rubus adenotrichus* (Acosta-Montoya et al., 2010; Gancel et al., 2010; Mertz et al., 2007), the USDA-ARS-Germplasm Resources Information Network (GRIN; www.ars-grin.gov) serves as a source for accurate *Rubus* botanical names.

Some researchers reported phenolic monomers after hydrolysis. For example, some researchers have intentionally hydrolysed blackberries and red raspberries (Jakobek et al., 2009; Mattila et al., 2006; Turkben et al., 2010), while others accidentally hydrolysed samples from harsh extraction procedures, and reported quercetin (Jakobek et al., 2009; Turkben et al., 2010), though quercetin is naturally found glycosylated in *Rubus* fruit (Mullen, Yokota, Lean, & Crozier, 2003; Maatta-Riihinen et al., 2004; Acosta-Montoya et al., 2010; Gancel et al., 2010; Mertz et al., 2007; Vasco et al., 2009). For reasons described in the following section, they may have overestimated native ellagic acid (Fig. 2A) content since ellagitannin is easily hydrolysed and changes form, thereby contributing to the concentration of this phenolic monomer fraction.

Additional work is needed for phenolic monomer clarification of *Rubus* fruit. When compared to the better-documented anthocyanins, it is clear that future work is required to further our understanding of *Rubus* fruit phenolic monomers. The phenolic monomer compounds in *Rubus* vary inter- and intra-specifically (references listed in Table 2), unlike the more definitive identifications of *Rubus* fruit anthocyanins.

2.3. Phenolic polymers

Phenolic polymers found in *Rubus* fruit are summarised in Table 3 and references therein. These compounds present a complex puzzle to decipher; they are extremely challenging to isolate, purify, and analyse. This phenolic group is frequently overlooked because of the difficulties in analysing them (Gasperotti et al., 2010; Salminen & Karonen, 2011), the limitations of available methods, and the lack of commercial standards. Despite extreme care given to storage, extraction, and processing procedures, even routine handling of samples prior to analysis (e.g. freezing, gentle extraction, or purification steps) can alter native polymer structures and degrade or breakdown the compounds under

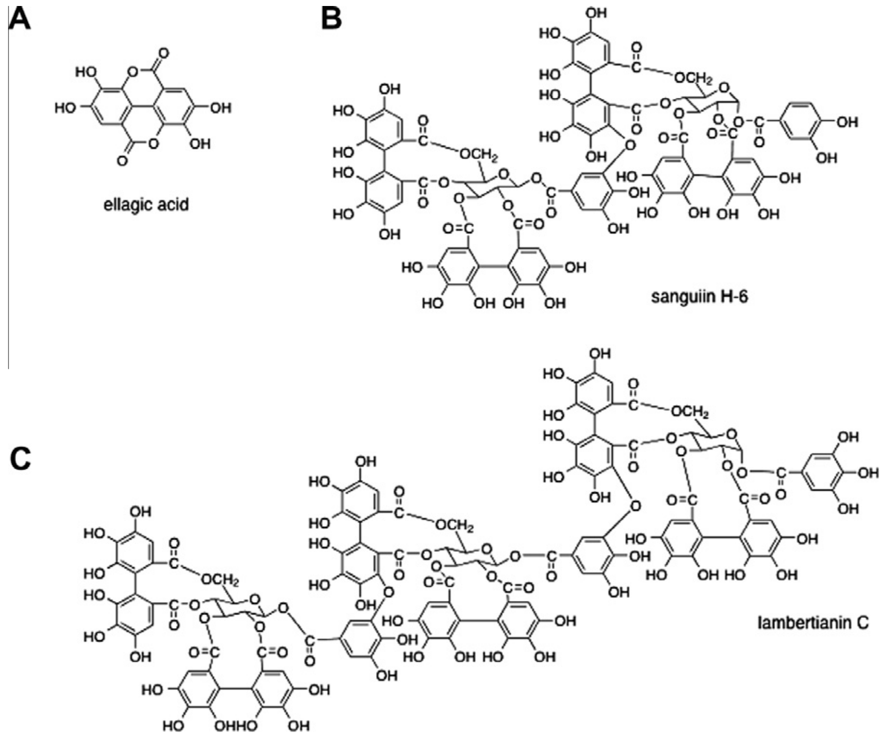


Fig. 2. (A) Ellagic acid, (B) sanguiin H-6, and (C) lambertianin C structures. These compounds are commonly found in *Rubus* fruit as summarised in this review.

observation (Hakkinen et al., 2000; Hager et al., 2010; Gasperotti et al., 2010; Salminen & Karonen, 2011).

Rubus fruit have been reported to contain ellagitannins (also referred to as hydrolysable tannins; ellagic acid derivatives), ellagic acid conjugates, and gallic acid conjugates (Gasperotti et al., 2010; Hager et al., 2008; Hakkinen et al., 2000; Kool et al., 2010; Maatta-Riihinen et al., 2004; Mertz et al., 2007; Remberg et al., 2010; Salminen & Karonen, 2011; Vasco et al., 2009; Vrhovsek et al., 2006). Hydrolysable tannins are complexes based on gallic acid or ellagic acid, and referred to as gallotannins or ellagitannins, respectively. A good review of ellagitannin chemistry and biology can be found in Quideau (2009). Clear examples of the UV–VIS spectra from ellagitannins, ellagic acid glycosides, and ellagic acid derivatives can be found in Maatta-Riihinen et al. (2004) and Arapitsas, Menichetti, Vinvieri, and Romani (2007).

The complexity of polymer molecules means they are typically quantified after acid hydrolysis and reported as their monomer building blocks, so in the case of ellagitannins they would be reported as simpler constituents like ellagic acid (Bushman et al., 2004; Hakkinen et al., 2000; Maatta-Riihinen et al., 2004; Mertz et al., 2007; Salminen & Karonen, 2011). When ellagitannin is hydrolysed it yields one or more hexahydroxydiphenic acids (HHDP), which rapidly lactonises to ellagic acid (Arapitsas et al., 2007; Koponen et al., 2007). HHDP fragmentation mass spectra can be found in Arapitsas et al. (2007). Recently, researchers have started to optimise a depolymerisation reaction (i.e. methanolysis) prior to examining its subunits by HPLC to provide a rough estimation of ellagitannin size (Hartzfeld, Forkner, Hunter, & Hagerman, 2002; Lei, Jervis, & Helm, 2001; Mertz et al., 2007; Vrhovsek et al., 2006, 2008). Vrhovsek et al. (2008) found 16 blackberry cultivars contained between 88 and 390 mg/100 g of ellagitannin (fw) with estimated mean degrees of polymerisation (mDP) ranging from 1.59 to 1.92. Their (Vrhovsek et al., 2006, 2008) methanolysis (optimised hydrolysis with 4 M HCl at 85 °C for 6 h of methanol extracts) of blackberries and raspberries produced subunits of methyl gallate, ellagic acid, methyl sanguisorboate, and an unknown ellagic acid derivative.

When intact ellagitannins were analysed in blackberries (*R. glaucus*, *R. adenotrichos*, and *R. fruticosus*) and red raspberries, sanguiin H-6 (dimer; co-eluted with lambertianin A in a few studies; Fig. 2B) and lambertianin C (trimer; Fig. 2C) were reported as the main ellagitannins (Acosta-Montoya et al., 2010; Borges et al., 2010; Mertz et al., 2007; Mullen, McGinn et al., 2002; Mullen, Stewart et al., 2002; Hager et al., 2010; Gancel et al., 2010; Gasperotti et al., 2010), with a combined concentration of 19–550 mg/100 g ellagitannins (fw; average 135 mg/100 g, $n = 18$). This was not the case for 'Boysenberry' however, as sanguiin H-6 and sanguiin H-10 (dimer) were reported as its main ellagitannins (Kool et al., 2010). Clear fragmentation mass spectra of sanguiin H-6 and lambertianin C can be found in Arapitsas et al. (2007). Whole tetramers (lambertianin D) have been reported in *Rubus* fruit (blackberries; Hager et al., 2008) and leaves (15 different species; Tanaka et al., 1993). Hager et al. (2008) and Hager et al., 2010 have reported finding additional hydrolysable tannins in blackberries (e.g. pedunculagin, castalagin, and vescalagin isomers) that are normally found in oaks (*Quercus robur* L. [syn. *Quercus pedunculata* Ehrh.] and *Quercus petraea* [Matt.] Liebl.) and chestnut (*Castanea sativa* Mill.) (Grundhofer, Niemetz, Schilling, & Gross, 2001; Peng, Scalbert, & Monties, 1991) that warrant confirmation by other research groups.

There are reports of *Rubus* fruit containing proanthocyanidins (also known as condensed tannins) (Beekwilder et al., 2005; Cuevas-Rodriguez et al., 2010; Furuuchi et al., 2011; Kellogg et al., 2010; Maatta-Riihinen et al., 2004; Vasco et al., 2009), but in reports with complete evaluations of *Rubus* fruit phenolics, the proanthocyanidins have been found in much lower concentrations than the ellagitannins (Beekwilder et al., 2005; Furuuchi et al., 2011; Vasco et al., 2009). Compared to black currants (*Ribes nigrum* L.), blueberries (*Vaccinium* spp.), bilberries (*Vaccinium* spp.), chokeberries (*Aronia* spp.), cranberries (*Vaccinium macrocarpon* Aiton), grapes (*Vitis vinifera* L.), or lingonberries (*Vaccinium vitis-idaea* L.), *Rubus* fruit are not rich in proanthocyanidins (Gu et al., 2004; Hellstrom, Torronen, & Mattila, 2009; Manach et al., 2004). Again, there is ample evidence that the major phenolic category within *Rubus* fruit are ellagitannins (Acosta-Montoya et al., 2010; Beekwilder et al., 2005; Gasperotti et al., 2010; Hager et al., 2008; Heinonen, 2007; Koponen et al., 2007; Laine, Kylli, Heinonen, & Jouppila, 2008; Maatta-Riihinen et al., 2004; Mertz et al., 2007; Mertz et al., 2009; Nohynek et al., 2006; Puupponen-Pimia et al., 2005; Remberg et al., 2010; Vasco et al., 2009), despite proanthocyanidin investigations that simply overlooked ellagitannins (Kellogg et al., 2010; Wada, 2009; Wu, Frei, Kennedy, & Zhao, 2010; Xu et al., 2006).

As ellagitannins are also the main phenolic compound in *Rubus* seeds (Bushman et al., 2004; Hager et al., 2008), research linking seed hardness, dormancy, or germination characteristics to phenolics (Wada, 2009) should not focus only on proanthocyanidins to the exclusion of ellagitannins. Wada (2009) attempted to make an association between seed proanthocyanidin concentration (0.45–2.81 μg/seed) and seed viability, but this link is not certain nor even complete if analysis did not include the dominant phenolic fraction. Collaboration between plant physiologists and phytochemists will aid in answering these links. As ellagitannin (a hydrolysable tannin) is the main phenolic in *Rubus* seeds and there are reports of hydrolysable tannins acting as an inhibitor for gibberellin promoted germination (Corcoran, Gessman, & Phinney, 1972), it is evident that more research on this topic is needed. Many *Rubus* seeds need chemical or physical scarification to germinate (Wada, 2009) and hydrolysable tannins may protect the dormant seed until germination (Corcoran et al., 1972).

Increased awareness of phenolic polymers can aid the pursuit of enhanced *Rubus* fruit phenolics. While breeding programs have customarily utilised phenological data, disease resistance, fruit maturity indices (pH, titratable acidity, and % soluble solids), fruit/berry size (or weight), firmness, flowering and fruiting dates, fruit yield, total anthocyanins by spectrophotometer, total phenolics by spectrophotometer, and individual anthocyanins by HPLC (Carew, Kempler, Moore, & Walters, 2009; Dossett et al., 2008, 2010; Finn & Knight, 2002; Jennings, 1988; Lewers, Wang, & Vinyard, 2010), we plan on including polymer analysis in future work. Since ellagic acid sediments can cause quality problems in 'Loganberry' wine (Singleton, Marsh, & Coven, 1966) and blackberry juice (Siriwoharn, Wrolstad, & Durst, 2005), new cultivars with decreased ellagitannin but maintained anthocyanins levels would benefit puree, fruit juice, and concentrate processors.

Again, ellagitannins are the main phenolic compounds found in *Rubus* fruit (Acosta-Montoya et al., 2010; Beekwilder et al., 2005; Gasperotti et al., 2010; Koponen et al., 2007; Maatta-Riihinen et al., 2004; Mertz et al., 2007; Nohynek et al., 2006; Okuda et al., 1992; Puupponen-Pimia et al., 2005; Vasco et al., 2009), making these an excellent source of dietary ellagitannins. It has been well established that these compounds are what makes fruit in the family Rosaceae unique (Bakkalbsai et al., 2009; Okuda, Yoshida, & Hatano, 2000; Okuda et al., 1992, 2005). Like the other phenolic groups previously discussed, there can be wide variation in *Rubus* fruit phenolic polymers among cultivars (Beekwilder et al., 2005; Gasperotti et al., 2010; Maatta-Riihinen et al., 2004; Siriwoharn & Wrolstad, 2004; Siriwoharn et al., 2004; Vrhovsek et al., 2008). *Rubus* fruit phenolic health benefits have been well reviewed by Rao and Snyder (2010). Additionally, phenolic polymers in *Rubus* fruit contribute to their potential health benefits by exhibiting antimicrobial (Beekwilder et al., 2005; Nohynek et al., 2006; Puupponen-Pimia et al., 2005), antiproliferative (Ross et al., 2007), antioxidative (Beekwilder et al., 2005; Mullen, Stewart et al., 2002; Ozgen et al., 2008; Patras, Brunton, Pieve, & Butler, 2009; Yokozawa et al., 1998), anti-inflammatory (Srivastava et al., 2010), and vasorelaxation (Mullen, McGinn et al., 2002) activities.

3. Concluding remarks

Rubus fruit anthocyanins have been frequently reported in the literature and peak identifications have been thoroughly confirmed by numerous research programs. Phenolics are a structurally diverse group of compounds and a single method cannot account for them all. Room remains for analytical method development, identification, and quantification of phenolic monomers and polymers other than anthocyanins. Additional work will also clarify the role ellagitannins have in *Rubus* plant development, a much needed area of research (Salminen & Karonen, 2011). *Rubus* phenolics are a fascinating and confusing group of compounds; much of their structurally diversity, polymer complexity, etc. will not be solved soon.

As many have pointed out (Dossett et al., 2008; McGhie et al., 2006; Tulio et al., 2008), correct phenolic identification in *Rubus* fruit is the first step to understanding their potential health benefits, which can guide cultivar selection, food processing conditions, product promotion, etc. Eventually, a complete understanding of phenolics in *Rubus* fruit will be revealed.

Acknowledgements

This project was funded by USDA-Agricultural Research Service (ARS) CRIS numbers 5358-21000-041-00D, 5358-21000-037-00D, and a grant funded by USDA-Northwest Center for Small Fruits Research Center (NCSFR, Corvallis, OR, USA).

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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