# The Blackberry Fruit: A Review on Its Composition and Chemistry, Metabolism and Bioavailability, and Health Benefits

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**ABSTRACT:** Blackberry (*Rubus* sp.) fruit contains high levels of anthocyanins and other phenolic compounds, mainly flavonols and ellagitannins, which contribute to its high antioxidant capacity and other biological activities. Blackberry phenolic composition and concentrations are known to be influenced by genetics, growing conditions, and maturation. Despite the current knowledge of their chemistry, research specific to blackberry phenolic compounds' health benefits, metabolism, bioavailability, and mechanism by which they confer health benefits is scarce. Blackberry phenolic compounds have protective effects on age-related neurodegenerative diseases and bone loss in vivo and can inhibit low-density lipoprotein and liposomal oxidation in vitro. Blackberry extracts have also exerted antimutagenic effects in vitro and in vivo by modifying cell signaling pathways and suppressing tumor promotion factors. However, the antiobesity, antidiabetic, antimicrobial, and anti-inflammatory properties of blackberry phenolic compounds need investigation. Similarly, studies that elucidate the in vivo physiologically effective concentrations of blackberry phenolic compounds are necessary.

**KEYWORDS:** blackberry, anthocyanins, bioavailability, flavonoids, health benefits

## ■ INTRODUCTION

Worldwide commercial production of blackberry (*Rubus* sp.) is estimated to be approximately 154,578 tons annually.[1] North America, Europe, Asia, South America, Oceania Central America, and Africa (in descending order of tons cultivated) are the main regions for blackberry production.[2] Wild blackberries are also cultivated in considerable amounts and in some regions may negatively affect the net sales of commercially cultivated fruit.[2]

The "berry" weighs from 3 to 12 g depending on variety and is best described as an aggregate fruit made up of several drupelets, each containing a seed.[1] Blackberries are mostly consumed fresh but can be processed and sold as individually quick frozen packs, bulk frozen, seedless or seeded puree, freeze-dried, or juice or concentrate.[1] In industry, blackberries are used for the production of dietary supplements, ice cream, jam, marmalade, and other confectionaries.[3]

Blackberry is a fruit of interest because of its high content of anthocyanins and ellagitannins (ETs) as well as other phenolic compounds that contribute to its high antioxidant capacity.[4] Several studies document the high antioxidant activity of blackberries based on their oxygen radical absorbance capacity (ORAC) compared to other fruits.[4] In fact, blackberries' medicinal qualities have been known since the 16th century in Europe, where they were used to treat infections of the mouth and eyes.[5]

Epidemiological and clinical studies[6−10] suggest that consumption of anthocyanins and other flavonoids found in most fruits and vegetables may decrease the risk of obesity, coronary heart disease, degenerative conditions, and various types of cancer. These health benefits and mechanisms by which anthocyanins confer them have been explored in vitro[7,11,12] and in animal models.[13−16] This review presents current knowledge on the chemistry, composition, metabolism and bioavailability, and health benefits of blackberries.

## ■ NUTRIENT COMPOSITION

Blackberry chemical composition varies on the basis of variety, growing conditions, stage of ripeness, and harvest and storage conditions.[17] In addition to valuable polyphenolic compounds, blackberries contain carbohydrates and several essential vitamins and minerals (Table 1).[18] The principal sugars in blackberries are glucose, fructose, and sucrose, and their ratios differ among cultivars.[19] The principal sugars are glucose and fructose in the ratios of approximately 3.24−2.88 and 0.81−1.17 g/100 g fresh weight (FW) glucose to fructose. Sucrose content is estimated to be 0.24 g/100 g FW.[19] Levels of glucose, fructose, total sugars, and total soluble solids increase markedly as the fruit ripens from light red to dark bluish purple stages.[20]

Blackberries contain malic acid as the primary organic acid, although different acids including ascorbic acid have been detected in the fruit.[3] Fan-Chiang[19] reported the following averages of nonvolatile organic acids from 52 samples of blackberry: 280 mg/100 g FW malic acid; 293 mg/100 g FW lactoisocitric acid; 599 mg/100 g FW isocitric acid; and 572 mg/100 g FW citric acid. Furthermore, trace amounts of shikimic, fumaric, and succinic acids were identified.[19] These organic acids in blackberries are important for stabilizing anthocyanins and ascorbic acid and extending the shelf life of fresh and processed berries.[17] The balance in proportion of organic and phenolic acids in berries is evaluated for overall index of fruit quality, whereas low pH is considered an indicator of poor quality.[17] Another important quality index for berries is the ratio of total soluble solids to total titratable acidity. This ratio

**Table 1. Chemical Composition of Blackberries from the U.S. Department of Agriculture Nutrient Database[18]**

| proximates and carbohydrates | | vitamin content | | mineral content | |
|---|---|---|---|---|---|
| water (g) | 88.20 | total ascorbic acid (mg) | 21.00 | calcium (mg) | 29.00 |
| energy (kcal) | 43.00 | thiamin (mg) | 0.02 | iron (mg) | 0.62 |
| protein (g) | 1.39 | riboflavin (mg) | 0.03 | magnesium (mg) | 20.00 |
| total lipids (g) | 0.49 | niacin (mg) | 0.65 | phosphorus (mg) | 22.00 |
| ash (g) | 0.37 | pantothenic acid (mg) | 0.28 | potassium (mg) | 162.00 |
| carbohydrate (g) | 9.61 | vitamin B6 (mg) | 0.03 | sodium (mg) | 1.00 |
| total fiber (g) | 5.30 | total folate ($\mu$g) | 25.00 | zinc (mg) | 0.53 |
| total sugars (g) | 4.88 | vitamin B12 ($\mu$g) | ND[a] | copper (mg) | 0.17 |
| sucrose (g) | 0.07 | vitamin A (IU) | 214.00 | manganese (mg) | 0.65 |
| glucose (g) | 2.31 | $\alpha$-tocopherol (mg) | 1.17 | selenium (mg) | 0.40 |
| fructose (g) | 2.40 | $\beta$-tocopherol (mg) | 0.04 | | |
| maltose (g) | 0.07 | $\gamma$-tocopherol (mg) | 1.34 | | |
| galactose (g) | 0.03 | $\Delta$-tocopherol (mg) | 0.90 | | |
| starch (g) | ND | vitamin K ($\mu$g) | 19.80 | | |

[a] ND, not detected.

**Table 2. Total Phenolics and Total Anthocyanin Values of Blackberries across Studies**

| total phenolics (mg GAE/100 g FW[a]) | total anthocyanins (mg/100 g FW) | no. of cultivars | source |
|---|---|---|---|
| NA[b] | 114–242[c] | 6 | 4 |
| 292–446 | NA | 6 | 23 |
| 248–633 | NA | 12 | 27 |
| NA | 70–201[c] | 51 | 28 |
| NA | 58–219[d] | 12 | 29 |
| 822–844 | 154–225[c] | 2 | 30 |
| NA | 80–230[c] | 27 | 31 |
| 418–555 | 111–123[c] | 2 | 32 |
| NA | 133–172[c] | 3 | 33 |
| 193–352 | 67–127[c] | 7 | 34 |
| 495–583 | 95–155[c] | 2 | 35 |
| 221–342 | 38–72[c] | 5 | 36 |
| 114–178 | 31–118[c] | 4 | 37 |
| NA | 83–326[c] | 16 | 38 |
| 682–1056 | 131–256[c] | 11 | 39 |

[a] FW, fresh weight. [b] NA, not available. [c] Calculated as cyanidin 3-glucoside equivalents. [d] Calculated as cyanidin 3-galactoside equivalents.

increases in blackberries as the fruit ripens from purple to dark bluish purple stages, reflecting the large increase in sugars and decline in organic acids.[20]

Blackberries contain cell wall hydrolase and oxidase enzymes, such as polyphenol oxidase and peroxidase, which contribute to the deterioration of quality in fresh, damaged, or pureed fruit.[17] Enzyme activity results in loss of texture, brown pigment formation, and destruction of phytochemicals that affects acceptability of, particularly, fresh fruit.[17] Refrigeration, reduced oxygen, addition of enzyme inhibitors, pH modification, and addition of reducing agents to control secondary oxidation are some of the techniques currently used to control enzyme activity.[17]

## PHENOLIC COMPOSITION

Blackberries are a rich source of polyphenolics including anthocyanins,[21] ETs,[22] flavonols,[23] flavan-3-ols, and procyanidins.[24]

The berries also contain appreciable levels of phenolic acids[25] and low levels of lignans.[26] Total phenolics in blackberries have been shown to range from 114 to 1056 mg/100 g FW (Table 2). Besides genetics, fruit maturity can influence levels of total phenolics in blackberries. For example, analysis of the berries of three blackberry cultivars, namely, 'Chester Thornless', 'Hull Thornless', and 'Triple Crown', demonstrated that total phenolic content significantly decreases as the fruit matures from the green to ripe stages.[33] However, in another study involving tropical highland blackberries, levels of total phenolics fluctuated as the fruit ripened from light red (580 mg/100 g FW) to purple (460 mg/100 g FW) to dark bluish purple (520 mg/100 g FW) stages.[20]

**Anthocyanins.** Anthocyanins are the phenolic compounds responsible for the red to purple to black pigments of fruits and vegetables[28] and are recognized for their potential health benefits.[7] Anthocyanins are flavonoids by classification and are predominantly found in berries and red grapes.[4] They are anthocyandinins glycosylated with one or more sugar moieties at the C3 position of the flavan structure.[25] Approximately 94% of blackberry anthocyanins occur in nonacylated form, and 90% of these exist as monoglycosides, whereas 10% are found as diglycosides[21] (Table 3).
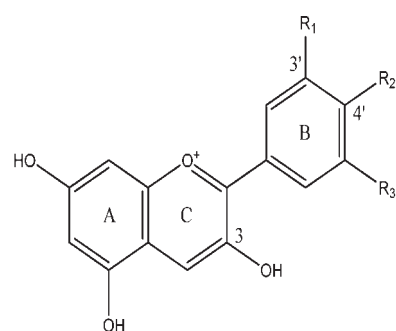
Blackberry anthocyanins are mainly cyanidin derivatives with glucose, rutinose, xylose, and arabinose moieties attached at C3[21,25] (Figure 1). Blackberry anthocyanins identified include cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-xyloside, cyanidin 3-dioxalyl-glucoside, cyanidin 3-rutinoside, cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-arabinoside, malvidin 3-arabinoside, perlargonidin 3-glucoside, cyanidin 3-(3-malonyl)glucoside, and cyanidin 3-(6-malonyl)glucoside.[40,41] Cyanidin 3-dioxaloylglucoside, a zwitterionic anthocyanin, is unique to blackberries.[42]

Studies show that the anthocyanin content of blackberry varies due to differences in variety, environmental conditions, cultivation site, degree of ripeness, and processing.[43] Cho et al. reported the total anthocyanin content of six blackberry genotypes to range from 114.4 to 241.5 mg/100 g FW.[4] In a similar study, Fan-Chiang and Wrolstad found monomeric anthocyanin content in 51 cultivars ranging from 70 to 201 mg/100 g FW, and the cyanidin derivatives dominated in all samples with a mean peak area of 83%.[28]

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**Table 3. Summary of Anthocyanins, Flavonols, and Ellagitannins Identified in Blackberries**[a]

| anthocyanins | flavonols | ellagitannins |
|---|---|---|
| cyanidin 3-galactoside | quercetin 3-galactoside | sanguiin H-6 |
| cyanidin 3-glucoside | quercetin 3-glucoside | lambertianin C |
| cyanidin 3-arabinoside, | quercetin 3-rutinoside | pedunculagin |
| cyanidin 3-xyloside | quercetin 3-xylosylglucuronide | lambertianin D |
| malvidin 3-arabinoside | quercetin 3-glucosylpentoside | galloyl-bis-HHDP glucose |
| perlargonidin 3-glucoside | kaempferol 3-glucuronide | sanguiin H-10 isomer (2) |
| cyanidin 3-rutinoside | kaempferol 3-glucoside | sanguiin H-6 minus gallic acid moiety |
| cyanidin 3-sophoroside | kaempferol 3-galactoside | lambertianin C minus ellagic acid moiety (4) |
| cyanidin 3-glucosylrutinoside | kaempferol 3-xylosylglucuronide | galloyl-HHDP glucose |
| cyanidin 3-(3-malonyl)glucoside | quercetin 3-glucuronide | sanguiin H-2 |
| cyanidin 3-(6-malonyl)glucoside | quercetin 3-O-[6-(3-hydroxy-3-methyl-glutaroyl)-$\beta$-D-galactoside | sanguiin H-6 plus gallic acid moiety |
| | myricetin | castalagin/vescalagin |
| | quercetin 3-methoxyhexoside | ellagic acid |
| | quercetin 3-oxalylpentoside | methyl ellagic acid pentose conjugate (2) |

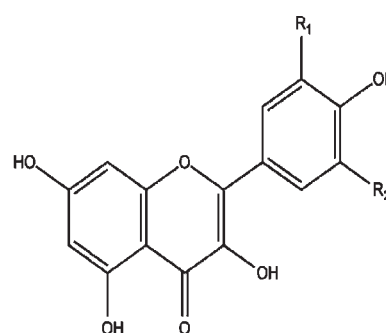[a] Compiled from the following references: 25, 32, and 52.



**Figure 1.** Chemical structures of blackberry anthocyanidins.

| Anthocyanidin | R$_1$ | R$_2$ | R$_3$ |
|---|---|---|---|
| Cyanidin | OH | OH | H |
| Pelargonidin | H | OH | H |
| Malvidin | OCH$_3$ | OH | OCH$_3$ |



**Figure 2.** Chemical structures of blackberry flavonols.

| Flavonol | R$_1$ | R$_2$ |
|---|---|---|
| Quercetin | OH | H |
| Kaempferol | H | H |
| Myricetin | OH | OH |

Moreover, cyanidin derivatives may vary in blackberries due to genetic differences.[28] This concept was demonstrated by Fan-Chiang et al.[28] using 51 samples of blackberries as follows: cyanidin 3-glucoside, 44−95%; cyanidin 3-rutinoside, trace amounts−53%; cyanidin 3-xyloside, undetectable amounts−11%; cyanidin 3-(malonyl)glucoside, trace amounts−5%; and cyanidin 3-dioxaloylglucoside, undetectable amounts−15%.[25,28]

Similarly, six blackberry genotypes analyzed by Cho et al.[4] demonstrated that distribution of cyanidin aglycones in each variety ranged from 75 to 84% for cyanidin 3-glucoside, from 1 to 12% for cyanidin 3-rutinoside, from 4 to 8% for cyanidin 3-dioxaloyglucoside, from 3 to 8% for cyanidin 3-dioxaloylgluco-side, and from 2 to 3% for cyanidin 3-(malonyl)glucoside.[4,25] Reyes-Carmona et al.[44] observed similar differences when comparing three cultivars: Marion, Evergreen, and Comanche berries. Unlike Marion and Evergreen, Comanche contained higher amounts of cyanidin 3-rutinoside than cyanidin 3-glucosides; however, Comanche's content of cyanidin 3-glucoside was still higher than those of Evergreen and Marion berries. Furthermore, malvidin 3-glucoside was identified in Marion and Evergreen, but not in Comanche berries.[44] Anthocyanins are synthesized during blackberry ripening, resulting in the development of a dark bluish purple color. Consistent with visual color change, total antho-cyanins increase markedly as the fruit ripens from pink/light red to dark bluish purple stages.[20,33]

**Flavonols.** The structure of flavonols entails a double bond at C2 and C3, a hydroxyl group at C3, and a ketone group at C4 of the C ring of the flavan nucleus[25] (Figure 2). Quercetin, myricetin, and kaempferol are the most commonly identified flavonols in berries; their main difference is the number and position of OH groups at C3 and C5.[45] The sugars commonly attached at the C3 position are glucose and galactose, but arabinose, rhamnose, rutinose, and xylose may also be found.[45]

Blackberries contain appreciable amounts of flavonols found exclusively in the fleshy part of the drupelet[30] and occur mostly in glycosylated form.[23] Reported values for total flavonols calculated as rutin equivalents are as follows: 12−18, 4−9 , not detectable−10, 10−16, 10−16, and 11−30 mg/100 FW.[4,23,30,32,35,46]

Blackberries have a complex flavonol profile due to the occurrence of nine quercetin and three kaempferol derivatives as well as two acylated quercetin-derived compounds (Table 3). In addition, quercetin 3-xylosylglucuronide, a different flavonol,

is reported in some blackberry varieties.[43] According to Cho et al.[23] quercetin 3-galactoside and quercetin 3-glucoside are the main flavonols in five blackberry genotypes, although a few varieties contain high levels of the acylated form of quercetin 3-[6-(3-hydroxy-3-methylglutaroyl)]-$\beta$-galactoside.[23] The occurrence of kaempferol and myricetin is not consistent for all blackberry genotypes, probably due to genetic differences.[4,39,47] Bilk and Sapers[48] reported that ripe fruits of thorn blackberry (*Rubus* spp.) contain 0.5–3.5 and 0.1–0.3 mg/100 g FW of quercetin and kaempferol, respectively,[48] whereas Sellappan and colleagues[32] reported 9.9 mg/100 g of myricetin in Georgia-grown blackberries. Levels of total flavonols in tropical highland blackberries are reported to decline as the fruit ripens from light red (30 mg/100 g FW) to dark bluish purple (14 mg/100 g FW) stages.[20]

**Phenolic Acids.** Phenolic acids in blackberry fruit range from 7 to 64 mg/100 g FW and are mainly hydroxybenzoic acid and hydroxycinnamic acids. These acids occur in conjugated forms of esters and glycosides, but rarely as free acids.[25] Esters make up 53.1% of total phenolic acids, whereas glycosides and free acids account for 43.6 and 3.3%, respectively.[49] Blackberry hydroxybenzoic acids include *p*-hydrobenzoic, protocatechuic, gallic, vanillic, salicylic, and gentisic acid. Glycosidic and ester forms of salicylic acid are the most common.[49]

Caffeic, *m*-coumaric, *p*-coumaric, and ferulic acid are hydroxycinnamic acids found in blackberries in free, ester, and glycosidic forms. The ester forms of *m*-coumaric, 3,4-dimethoxy cinnamic, and hydroxycaffeic acids are predominant.[49] Blackberry hydroxybenzoic and hydroxycinnamic acid derivatives have been classified as follows: chlorogenic acid, neochlorogenic acid, and glucose esters of caffeic, *p*-coumaric, ferulic, and gallic acids, as well as protocatechuic acids and the $\beta$-D-glucosides of *p*-coumaric and *p*-hydroxybenzoic acids.[50]

Ellagic acid (EA) is a hydroxybenzoic acid, but most of the EA found in blackberries is in the form of ETs, which is a different class of phenolics. Blackberry EA calculated as "ellagic acid equivalents" following acid hydrolysis has been reported in several studies to range from 2 to 4 mg/100 g FW,[30] from 2 to 34 mg/100 g FW,[49] from 12 to 18 mg/100 g FW,[46] from 21 to 25 mg/100 g FW,[37] from 30 to 34 mg/100 g FW,[32] and frpm 59 to 90 mg/100 g FW.[35] Ellagic acid derivatives are reported to decline as blackberries ripen from light red (30 mg/100 g FW) to dark bluish purple (20 mg/100 g FW) stages.[20]

**Tannins.** Tannins are a group of polyphenolic compounds found in many berry fruits and include oligomeric and polymeric constituents.[41] Tannins are known to be antinutritional because the tannins bind to —NH groups of peptides, precipitate them, and prevent their hydrolysis in the stomach.[41] For example, flavan-3-ols (catechins) and flavan-3,4-diols (leucoanthocyanins) interact with proteins, starch, and digestive enzymes to form less digestible complexes, resulting in reduced absorption of these nutrients.[51]

Tannins are classified into two groups on the basis of their structure: condensed tannins, that is, proanthocyanidins, and hydrolyzable tannins, that is, ETs and gallotannins.[41] Proanthocyanidins are oligomers and polymers of flavonoids and in particular flavan-3-ols, whereas the hydrolyzable tannins are glycosylated gallic acids.[41] Berry fruits contain two major types of proanthocyanidins: procyanidins and propelargonidins. The procyanidins and propelargonidins are composed exclusively of (epi)catechin and (epi)afzelchin units, respectively.[25] On the basis of the hydroxylation pattern of A- and B-rings, proanthocyadinins (MW ≥ 2000−4000 Da) are categorized into three groups: procyanidins, prodelphinidins, and propelargonidins.[41]

There are about 50 procyanidins identified from plants.[41] Procyanidins in blackberries have been found to be predominantly epicatechin units linked through a C4→C8 bond, the so-called "B-type" linkage, with an average degree of polymerization of 3.2.[24] Blackberries contain monomers (7.3 mg/100 g FW), dimers (6.7 mg/100 g FW), trimers (3.6 mg/100 g FW), tetramers to hexamers (7.3 mg/100 g FW), heptamers to pentamers (4.2 mg/100 g FW), and low levels of polymers (1.5 mg/100 g FW), resulting in a total procyanidin content of 27.0 mg/100 g FW.[24] Hydrolyzable tannins, specifically ETs of various molecular weights, are found in blackberries in appreciable amounts.[25] Ellagitannin and EA derivatives are identified using high-performance liquid chromatography—mass spectrometry (HPLC-MS), and results are commonly reported as "ellagic acid equivalents" due to the diversity of ETs in berries and lack of ellagitannin standards.[25]

Blackberry ET structures are complex, large, and diverse, which presents a challenge in their characterization[52] (Table 3). The basic structure of ETs consists of a glucose core esterified with hexahydroxydiphenic acid (HHDP) (Figure 3). When hydrolyzed by either an acid or a base, HHDP is changed to the dilactone form, EA.[25] An ET bis-HHDP glucopyranose is also known as pedunculagin, and the galloylated form, or galloylbis-HHDP glucopyranose, is also known as casuarictin or potentillin.[52] Moreover, these compounds can be found as $\alpha$- or $\beta$-glucopyranosides[53] with the HHDP units R or S configurations relative to C4 and C1 of the glucose.[54] ETs found in blackberry fruit and leaves are mostly polymerized forms of the galloyl-bis-HHDP.[52]

Blackberry seeds contain the highest amount of ETs and EA compared to other parts of the fruit, that is, the torus and flesh.[30] In fact, 88% of the ETs and EA is found in the seeds compared to only 12% in the pulp. EA derivatives in blackberries range from 1.2 to 3.0 mg/100 g, whereas ETs range from 51.1 to 68.2 mg/100 mg.[46] The seeds of Marion and Evergreen blackberry cultivars have higher ellagitannin values than most cultivars, containing up to 3230 and 2120 mg/100 g seed of total ellagic acid, respectively.[22]

Recently, blackberries were investigated by high-performance liquid chromatography—electrospray ionization—mass spectrometry and matrix-assisted laser desorption/ionization—time of-flight mass spectrometry (MALDI-TOF-MS) to identify the ETs.[52] The following 11 ETs were identified mainly in the seed and torus of the Apache cultivar of blackberries: isomeric forms of pedunculagin, castalagin/vescalagin, galloyl-HHDP glucose, lambertianin C, lambertianin D, and galloyl-bis-HHDP glucose, sanguiin H-6/lambertianin A, and EA.[52] This study also proposed that blackberry fruit may contain sanguiin H-10.[52] In another recent study, ETs and EA conjugates in six blackberry cultivars were identified and quantified using ultrahigh-pressure liquid chromatography—quadrapole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS) and HPLC-DAD analyses.[55] In this study the authors isolated and purified the two major ETs found in blackberries, lambertianin C and sanguiin H-6, and used them as calibration standards. Besides lambertianin C and sanguiin H-6, which account for 67% of the total ETs, they identified 12 additional ETs, as well as EA and 2 EA conjugates. The levels of total ETs in the six cultivars ranged from 84.7 to 130.4 mg/100 g FW, whereas total EA conjugates ranged from 12.8 to 27.4 mg/100 g FW. The ETs potentillin, pedunculagin, and casuaricitin have also been identified in leaves and shoots of blackberries.[56] Notably, ET and ellagic content of blackberries is affected by maturation. For instance, in the tropical highland blackberry cultivar, *Rubus adenotrichus*, known to have the highest amount of ETs in an edible fruit when light red (380 mg ellagic acid equiv/100 g of fresh
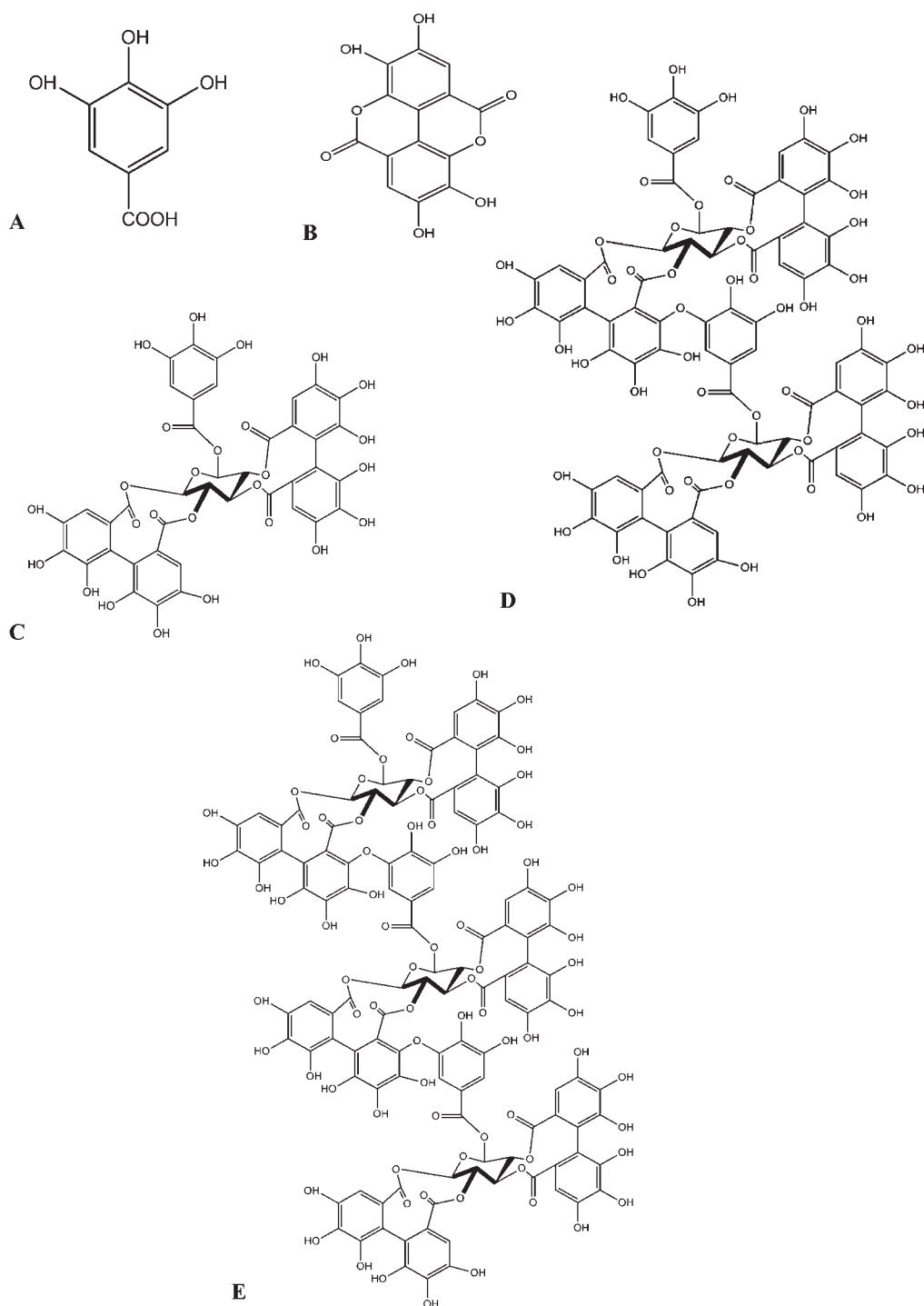
**Figure 3.** Structures of gallic acid (A), ellagic acid (B), galloyl-bis-HHDP glucose (C), sanguiin H-6/lambertianin A (D), and lambertianin C (E). Structures adapted from ref 52.

fruits), the two major ETs, lambertianin C and sanguiin H-6, decline 33% from light red to dark bluish purple stages.[20]

## OTHER PHYTOCHEMICALS

**Lignans.** Lignans are biphenolic compounds recognized as phytoestrogens and play an important role in the prevention of hormone-associated cancers such as breast cancer[57−59] and other conditions including cardiovascular disease[60,61] and osteoporosis.[62]

Blackberries are unique as they contain both secoisolariciresinol (3.72 mg/100 DW) and matairesinol (<0.01 mg/100 g DW).[26] These lignans are known to be converted to mammalian lignans enterolactone and enterodiol by gut microflora.[63]

## METABOLISM AND BIOAVAILABILITY

Although phytochemicals have been studied extensively, there is insufficient information regarding changes in the content and

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character of polyphenolic compounds once consumed.[64] In general, bioabsorption of anthocyanins, which are the most studied and abundant flavonoids, occurs very quickly after consumption, appearing in plasma 15–60 min postprandial, and excretion is complete within 6–8 h.[65−73] Hollman et al.[74] noted that glycosylation of flavonols and their derivatives influence their metabolism, absorption, and bioavailability in vivo. Following absorption, anthocyanins are metabolized differently on the basis of their aglycones via methylation, glucuronidation, and sulfoconjugation.[69,70,75−77] This pathway uses conjugations with glucuronic acid or with sulfate and is considered to be important in the detoxication pathway of many drugs and xenobiotic substances.[67] These processes are catalyzed by UDP-glucuronosyl transferase and sulfotransferase, respectively, in the small intestine, liver, and/or kidney.[67] Some animal[78,79] and human studies[73,80] are consistent with these pathways as glucuronide forms of different subclasses of flavonoids have been found in plasma or urine.[81,82]

Anthocyanins are absorbed in intact glycoside forms from the digestive tract into the blood circulation system in mammals.[83] Although some argue that anthocyanins are too hydrophilic to be absorbed into cells of animals and humans,[84] there are studies[85,86] that support this pathway for small molecular compounds such as cyanidin-3-glucoside. Other polyphenolic compounds such as free simple phenolic acids, aglycones, can also be metabolized in the gut and absorbed by enterocytes.[87−89] Anthocyanins have also been reported to be substrates of bilitranslocase, which is a plasma membrane carrier involved in organic anion uptake and mainly expressed at the sinusoidal domain of the liver plasma membrane and in epithelial cells of the gastric mucosa.[86] Using in situ exposure to tissue, investigators have found that absorption occurs in the stomach, jejunum, and duodenal tissue, possibly through interaction with bilitranslocase.[65,85,90]

Anthocyanins are also thought to be metabolized via intestinal microflora through cleavage of the C-ring to produce a range of phenolic acids that are easily absorbed.[75] Previously, scientists thought that the absorption of glycosides was not possible in mammals due to the lack of suitable $\beta$-glycosidases,[91] but light has been shed on the role of glycosidases from gut microflora in the ileum that aid in the metabolism and absorption of glycosides such as quercetin glycosides.[88,92] However, this mechanism is unclear because the gut pH affects the stability of individual flavonoids and especially anthocyanins, which actually results in increased recovery of intact compounds in feces.[68,79,93] Complex anthocyanins containing di- or triglycosides are reported to remain in the gut longer than simple anthocyanins (e.g., monoglycosides); consequently, plasma total antioxidant capacity and total anthocyanins remain high longer after feeding.[68] It has been suggested that it is by this mechanism that anthocyanins provide significant antioxidant protection in the environment of the gut epithelium.[68]

Studies on the metabolism of blackberry cyanidin glycosides in particular are limited. In general, cyanidin glycosides are absorbed intact in the gut or conjugated with glucuronide or methylated in the liver or gut and may be either excreted into the bile directly or enter blood circulation and excreted in urine.[67,80,94] The conjugated compounds identified so far include peonidin monoglucuronide, peonidin-3-glucuronide, and cyanidin 3-glucoside monoglucuronide.[67] Glucuronidation of cyanidin 3-glucoside has also been established in vivo and in vitro.[67,81] It has been suggested that although all four free hydroxyl groups of cyanidin have different glucuronidation capacities, glucuronidation of cyanidin-3-glucoside

occurs most readily at the 3-position.[67,81] Quercetin glycosides, which are also abundant in blackberries, are converted to glucosides, resulting in rapid absorption possibly via glucose transporters in the gut.[92,95−97]

Systemic bioavailability of anthocyanins in general is very low across all studies, often between 0.02 and 1.8% of the ingested amounts, although as low as 0.004−0.11% of administered dose has been reported.[75] A human study by Felgines and others[69] fed 200 g of blackberries containing 960 $\mu$mol of anthocyanins to five healthy volunteers and found that only 0.16% of the total amount of anthocyanins ingested was excreted in urine, mainly in the form of methylated and glucuronidated conjugates.[69] The study identified cyanidin 3-glucoside, methylated glycosides, glucuronides of anthocyanidins and anthocyanins, and sulfoconjugates of cyanidin and anthocyanidins. Monoglucuronides of anthocyanidins accounted for >60% of the total anthocyanins in the urine.[69] Findings on the metabolism and bioavailability of polyphenolic compounds may vary due to differences in animal models and experimental designs. Additionally, these compounds have different chemical structures, and solubility can interact with macro- and micronutrients; they also vary in susceptibility to digestion, conjugation, methylation, fermentation, and absorption in the gut.[91]

Studies pertinent to the metabolism and bioavailability of blackberry ETs and EA are lacking. However, this section will briefly highlight the metabolism and bioavailability of various other ETs and EA found in the literature and hypothesize that the pathways may be similar.

A study on punicalagin, an ET found in both blackberries and pomegranate, was detectable in human plasma and urine after consumption of 180 mL of pomegranate juice.[98] The juice contained 25 and 318 mg of EA and ETs, respectively. The highest concentration of EA in human plasma 1 h postprandial was 31.9 ng/mL, and EA was eliminated within 4 h.[98] The authors hypothesized that ETs and EA were metabolized by the colon microbiota, hence the appearance of free EA in human plasma.[98] In rats, ETs are hydrolyzable to EA by gut microbiota in the small intestine and cecum.[99] EA fed to rats was detected in the form of urolithin A, also known as 3,8-dihydroxy-6*H*-dibenzo[*b*,*d*]pyran-6-one, and an unidentified metabolite in urine and feces. Investigators detected as much as 10% of the given dose in this study.[100] Further work using germ-free animals showed that these metabolites resulted from action by gut microorganisms.[100] EA can also be metabolized via conjugation, possibly in the liver or in the gut as evident by two conjugates of urolithin A found in rats after ingestion of EA.[100] A mouse study using phenol EA ($^3$H-EA) also detected free EA and conjugates in urine, bile, and blood and after gavage, and 80% of the fecal sample fraction was free EA.[101] A study using Iberian pigs demonstrated that ETs release EA in vivo followed by a gradual metabolism of and production of urolithins D, C, A, and B in this order from the jejunum to the distal portion of the intestine.[102] Furthermore, absorption of these metabolites was found to increase with their increasing lipophilicity.[102] Therefore, ETs and EA are metabolized by the gut microbiota of different mammals,[98,102,103] resulting in dibenzopyranones, urolithin A, and its monohydroxylated analogues urolithins B, C, and D.[104] It is thought that ETs are hydrolyzed to EA due to the intestinal pH and or action by gut microflora, and EA is transformed to urolithins A, B, C, and D by gut microflora through lactone-ring cleavage via decarboxylation and dehydroxylation reactions.[102] The Iberian pig study also confirmed enterohepatic circulation

activity as glucuronides and methyl glucuronides of EA and urolithin A, C, and D derivatives were detected in bile and urolithins A and B and dimethyl-EA glucuronide were found in peripheral plasma.[102]

Because it takes 12−56 h to complete excretion of urolithins and their metabolites, their distribution in tissues such as liver, lung, kidney, heart, muscle, and adipose tissue has been investigated. No such metabolites were detected in these tissues in pigs,[102] although traces of punicalagin metabolites were reported in the kidney and liver of rats[103] and in the colon, intestine, and prostate tissues of mice.[105]

In summary, gut microbiota are responsible for hydrolysis of ETs to EA and subsequently to glucuronides and methyl glucuronides of EA, urolithins A, B, C, and D, and urolithin derivatives. It has been suggested that differences exist in the metabolism of ETs and EA on the basis of food sources and host colonic microflora composition that result in variation in the timing, quantity, and types of urolithins absorbed and excreted in urine.[106] Therefore, metabolism and bioavailability of particular blackberry ETs and EA require investigation as a step toward exploring their health benefits.

## ■ HEALTH BENEFITS

Anthocyanins are reported to exert anti-inflammatory, antiviral, antiproliferant, and anticarcinogenic properties.[51] The consumption of anthocyanins in the United States is generally higher than that of other flavonoids due to their widespread distribution and occurrence in fruits and vegetables[65,107] and is estimated to be between 82 and 215 mg/day. Presently, data on dietary intake of blackberry ETs and EA are lacking. However, as a reference, daily dietary intakes of ETs from berries in the Finnish and German populations are 12 and 5 mg/day, respectively.[108,109] Health benefits of particular blackberry ETs and EA have also not been explored, although a recently published review of ETs and EA is detailed on the anti-inflammatory, antimicrobial, prebiotic, antioxidant, and estrogenic and/or antiestrogenic of dietary ETs and EA.[110]

**Oxidative Stress (OS).** Living organisms have a reduction−oxidation system that is necessary to maintain a balance between free radicals generated and the antioxidant system.[75] The formation of large amounts of free radicals may cause OS, leading to many degenerative diseases and aging.[75,111−113] Although some scientists argue that in many instances OS is not the primary cause of disease and that the formation of radicals is secondary to tissue damage by disease,[51] there is some evidence linking OS with several chronic diseases.[114,115]

Wada and Ou[35] ranked the antioxidant activity of blackberries third highest after strawberry and black raspberry on the basis of their ORAC.[35] This can be attributed to the high amounts of acylated anthocyanins and cyanidin 3-glucoside in blackberries. In fact, cyanidin-3-glucoside is ranked high in ORAC activity and is reported to be 3.5 times stronger than Trolox (vitamin E analogue), whereas pelargonidin, also found in blackberries, was reported to have antioxidant activity equivalent to that of Trolox.[107] Acylated anthocyanins are highly correlated with antioxidant capacity in fruit.[4] Cho et al. observed a strong linear relationship between ORAC values and total acylated anthocyanins ($r_{xy}$ = 0.91) in blackberry extracts, and this correlation was weaker for total anthocyanin monoglucosides ($r_{xy}$ = 0.69).[4] This correlation is consistent with bioavailability studies that demonstrate structural differences in anthocyanins such as sugar

### Table 4. ORAC Values of Blackberries[a]

| fresh weight ($\mu$mol Trolox equiv/g) | dry weight ($\mu$mol Trolox equiv/g) | no. of cultivars | source |
|---|---|---|---|
| 63−83$_{FL}$ | | 6 | 4 |
| 49−76$_{FL}$ | | 6 | 23 |
| 34−36$_{PE}$ | | 2 | 30 |
| 27−71$_{PE}$ | | 27 | 31 |
| 20−25$_{PE}$ | 121−146$_{PE}$ | 3 | 33 |
| 28$_{PE}$ | | 2 | 35 |
| | 334−560$_{FL}$ | 11 | 44 |
| 38−76$_{PE}$ | | 11 | 39 |
| 17−67$_{FL}$ | | 15 | 117 |
| 43−62$_{FL}$ | | 4 | 118 |

[a] FL represents ORAC results using fluorescein probe, whereas PE represents ORAC results using B-PE probe.

moieties, and acylated groups affect bioavailability, stability, and other biological effects of anthocyanins.[21] It should be noted that biological activities of berry components may vary with species and even cultivar.[75]

Anthocyanins work by scavenging free radicals, with a 3, 4-dihydroxy substituent in the B ring[75] being a key criterion for radical scavenging. They react readily with radicals such as hydroxyl ($^\bullet$OH), azide ($N_3^\bullet$), and peroxyl ($ROO^\bullet$) to form stable flavonoid radicals,[51] hence reducing OS.[116] Flavonoids, however, are slower at reacting with lipid peroxl ($LOO^\bullet$) radicals and superoxide ($O_2^-$) due to reduced availability of polar flavonoids for reaction with the $LOO^\bullet$ radicals.[116] They also enhance fatty acid stability by reacting with $\alpha$-tocopherol radicals to form $\alpha$-tocopherol.[51,116] Anthocyanins also function as antioxidants by chelating metal ions at moderate pH levels with their ionized hydroxyl groups of the B ring.[75]

Several studies have examined the ORAC of blackberries, and results are presented in Table 4. When ORAC results by different authors are compared, it should be noted that the use of fluorescein probe results in higher ORAC values, typically 1.5−3.5-fold, compared to older studies that used the B-PE probe.[119]

**Cardiovascular Disease (CVD).** Elevated levels of low-density lipoproteins (LDLs) in plasma are an independent risk factor for CD.[120] Berry phenolic compounds have shown protective effects against CD by inhibiting in vitro and in vivo oxidation of LDL by quenching free radicals through donation of hydrogen molecules.[41] Berry phenolic compounds also protect LDL from hydrogen peroxide-induced oxidative stress in human endothelial cells in vitro.[75]

Blackberry phenolic compounds demonstrated strong inhibitory properties in vitro using oxidation assays on human LDL and lecithin liposomes.[121] High contents of cyanidin glycosides in blackberries are responsible for the high antioxidant activity and protection against LDL oxidation, whereas the hydroxycinnamic acids are most important in the liposome oxidation system.[121] Anthocyanins, flavan-3-ols, and hydroxycinnamic acids have been shown to have inhibitory effects in liposomal oxidation.[121] Furthermore, anthocyanins have exerted protective effects in vitro on human primary endothelial cells by suppressing the secretion of cytokine-induced chemokine monocyte chemotactic protein 1 (MCP-1).[72] MCP-1 is a protein directly involved in atherogenesis through its role of recruiting macrophages to sites of infection or inflammation.[72,122]

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Although not demonstrated directly by blackberry flavonoids, select flavonoids have been shown to play a protective role in platelet function, which is critical in the pathogenesis of CD.[123] In vitro studies have shown that flavonoids reduce platelet aggregation, decrease platelet production of superoxide anions, and increase platelet nitric oxide production.[123]

**Cancer.** Evidence from epidemiological and clinical studies suggests that 20% or more of all cancer cases are preventable with a diet consisting of 400−800 g of various vegetables and fruits per day.[75] Cancer is a complex multistage process that begins with initiation of a cancer cell caused by DNA damage, accumulation of mutations, promotion of cell proliferation and tumor expansion, and finally progression to malignancy and metastasis.[124] The potential role of berry phenolic compounds to reduce cancer risk has been shown in in vitro, animal, and clinical studies.[75] Berry phytochemicals may act to change the genomic stability of cells at several points along this sequence of cancer formation.[124] The phytochemicals may modulate initiation, promotion, and progression of cancer.[124] The possible anticarcinogenesis mechanisms include antioxidant activity, detoxification activity, induction of apoptosis, antiproliferation, and antiangiogenic activity.[75] Anthocyanins have been reported to induce phase II enzymes, which may inactivate carcinogens activated by phase I enzymes, therefore inhibiting possible DNA damage by the carcinogens.[75] Tate et al.[125] found that blackberry extracts from eight varieties (Arapaho, Choctaw, Hull, Chicksaw, Triple Crown, Kiowa, Navajo, and Chester) suppressed mutagenesis at various levels. Mutagenesis is a process characterized by uncontrolled cell proliferation and resistance to programmed cell death.[124] Furthermore, blackberry extracts prepared from eight varieties inhibited UV-induced mutagenesis in *Salmonella typhimurium* TA100 by 90% based on the Ames test.[125] The Ames test is a good in vitro indicator of mutagenic potential in vivo, with a 90% correlation between a positive response in assay and carcinogenicity in animal models.[125]

Serraino et al.[126] reported that blackberry extracts play a protective role against peroxynitrite-induced DNA strand breakage in cultured human vascular endothelial cells. Using blackberry extracts containing 80% cyanidin 3-glucoside, the authors investigated the antioxidant activity of the blackberry extract on the endothelial dysfunction in cells and in vascular rings exposed to peroxynitrite. Blackberry extracts at various dilutions reduced the peroxynitrite-induced suppression of mitochondrial respiration and DNA damage in human umbilical vein endothelial cells.[126]

In addition, in vitro studies show that blackberry extract inhibits proliferation of A549 human lung cancer cells and reduces neoplastic transformation in normal epidermal JB6 mouse cells exposed to the phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) tumor promoter.[124] Blackberry anthocyanins are thought to suppress cancer cell growth by modifying cell signaling pathways such as modulating the expression of activated protein 1 (AP-1) and nuclear factor κB (NFκB), important proteins that regulate cell proliferation and cell cycle control.[124] Blackberry extracts have also demonstrated inhibitory properties in cancer-induced AP-1 and NFκB and suppressed the expression of the two proteins involved in tumor promotion and progression, vascular endothelial growth factor and COX-2.[124] Moreover, Seeram et al.[127] have demonstrated the dose-dependent cancer inhibitory properties of blackberry extracts in vitro in a study that used human oral, breast, prostate, and colon cancer cell lines. Similarly, a study using quercetin extracted from blackberries also demonstrated the anticarcinogenic properties in animal models and in human carcinoma cell lines (HT29 and Caco-2).[75,128]

**Other Health Benefits.** Studies on blackberries and their effect on body weight are lacking. However, cyanidin 3-glycoside (which is the predominant anthocyanin in blackberries) from purple corn has been shown to prevent obesity in C57BL/6J mice fed a high-fat diet compared to mice fed a high-fat diet with no anthocyanins.[16] Similarly, purified blueberry anthocyanins have been shown to improve body weight and body composition and reduce obesity in mice.[129] Although matching health benefits may be expected from blackberry anthocyanins, their effects on obesity need to be explored.

Blackberries are reported to have positive effects on age-related changes and may be beneficial for prevention of age-related neurodegenerative diseases such as Alzheimer's disease.[15] Shukitt-Hale and others demonstrated that 2% blackberry supplemented in the diet of 19-month-old Fischer rats was effective in improving motor performance on three balance and coordination tasks and cognitive performance.[15] Cyanidin 3-glucoside from blackberries was found in the brain of male Wistar rats after 15 days of feeding a 1.5% blackberry diet,[130] supporting the potential role of blackberry anthocyanins in neuroprotection.

Although the antimicrobial activities of blackberry tannins are not documented, hydrolyzable tannins from red raspberries have shown antimicrobial activities on *Staphylococcus* and *Salmonella* bacteria in animal studies.[75] Because blackberries provide a rich source of ETs, their antimicrobial properties should be investigated.

Blackberry anthocyanins in particular have not been studied in relation to diabetes; however, there is evidence that in general anthocyanins can confer protective effects to the vascular system in diabetic patients. Administration of anthocyanins from bilberry (*Vaccinium myrtillus*) at a dose of 600 mg/day for 6 months demonstrated significant reduction of the biosynthesis of polymeric collagen and structural glycoprotein that result in capillary thickness in diabetes.[131]

A study by Kaume and others[132] has recently demonstrated that supplementation of blackberries at the level of 5% but not 10% in ovariectomized rats modestly increased bone mineral density at the tibia by 2.4%, at the femur by 4.3%, and at the fourth lumbar vertebra by 2.7% higher than ovariectomized control animals ($P < 0.05$). Furthermore, animals fed 5% blackberry (w/w) had a significant decrease in trabecular separation, by 22% less than the ovariectomized control rats. More studies on the health benefits of blackberries on bone are necessary to elucidate the mechanism by which these berry compounds modulate bone metabolism.

Studies on blackberries reveal that their phenolic composition and concentration are influenced by many factors including variety, location of cultivation, and maturation. The chemistry of blackberry phenolic compounds is well understood. In terms of health benefits, these phenolic compounds have shown protective effects in age-related neurodegenerative diseases and bone loss in vivo and inhibited LDL and liposomal oxidation in vitro. Blackberry extracts have also exerted antimutagenic effects by modifying cell signaling pathways and suppressing tumor promotion factors in vitro and in vivo. However, the antiobesity, antidiabetic, antimicrobial, and anti-inflammatory properties of blackberry phenolic compounds including catabolites from colonic microflora need investigation. In addition, more research is needed to explore their metabolism and bioavailability and the

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mechanisms by which they confer health benefits. Furthermore, studies that elucidate in vivo physiologically effective concentrations of blackberry phenolic compounds in vivo are necessary.

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