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Chapter 6 Cranberry

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6.1 CRANBERRY: INTRODUCTION AND TRADITIONAL ORIGINS

The name “cranberry” reportedly derives from the Pilgrim name for the fruit “craneberry,” because the small, pink blossoms that appear in the spring resemble the head and bill of a Sandhill crane. Cranberries are unique among fruits. They can grow and survive only under a special combination of factors. They require acid peat soil, adequate freshwater supply, sand, and a growing season that stretches from April to November and is followed by a period of dormancy in the winter months that provides an extended cold period, necessary for fruiting buds to mature. Contrary to popular belief, cranberries do not grow in water. Instead, they grow on vines in impermeable beds that are layered with sand, peat, gravel, and clay. These beds, commonly known as “bogs,” were originally made by glacial deposits, but now, they can be made by humans. Growers do not have to replant, since an undamaged cranberry vine will survive indefinitely. Some vines in Massachusetts are more than 150 years old. European settlers adopted the Native American uses of the fruit and found that cranberry is a valuable bartering tool. American whalers and mariners carried cranberries on their voyages to prevent scurvy. In 1816, Captain Henry Hall was the first to successfully cultivate cranberries (Cape Cod Cranberry Grower’s Association, http://www.cranberries.org/cranberries/history.html). Today, cranberries are available in both fresh and processed forms. There are many varieties of cranberry fruit; white cranberry is an early harvest cranberry, which is picked 2–3 weeks prior to ripening.

There are two major species of cranberry: the American cranberry (Vaccinium macrocarpon) and the European cranberry (V. oxycoccos). The European cranberry fruit is smaller (0.6–1.2 cm) and only half the size of the American fruit. The American cranberry, which is frequently cultivated, is a member of the Ericaceae family, evergreens, creeping shrubs native to the cool, temperate, acidic soils and peat wetlands of Northeastern United States and southern Canada. Latvia, Belarus, Azerbaijan, and Ukraine are other cranberry-producing countries in Europe, with Turkey just beginning cranberry cultivation. The United States and Canada together account for more than 90% of the world’s production (Zhao 2007). The forecast for U.S. cranberry production in 2010 is 735 million pounds up by 6% from 2009 figures and the second highest yearly production. Wisconsin is expected to lead all U.S. states in the production of cranberries, with 435 million pounds, followed by Massachusetts, with 195 million. New Jersey, Oregon, and Washington are also expected to have substantial

production (Cranberries, National Agricultural Statistics Service (NASS), Agriculture Statistics Board, United States Department of Agriculture (USDA), August 17, 2010).

Cranberries are low-growing, woody, perennial vines with small, alternate, and ovate leaves. The plant produces stolons (horizontal stems) having a height of up to 6 feet (2 m). Short, vertical branches, or uprights, 2–8 inches (5–20 cm) in height, grow from buds on the stolons, and these can be either vegetative or fruiting. Each fruiting upright may contain as much as seven flowers. Pollination is primarily via domestic honeybees (Cranberry Institute, East Wareham, Massachusetts). Cranberries were first used by Native Americans, who discovered the wild berry's versatility. Native Americans used cranberries in a variety of foods, the most popular being pemmican, a high-protein combination of crushed cranberries, dried deer meat, and melted fat. They also used it as a medicine to treat arrow wounds and as a dye for rugs and blankets.

6.2 PRODUCTION AND CONSUMPTION

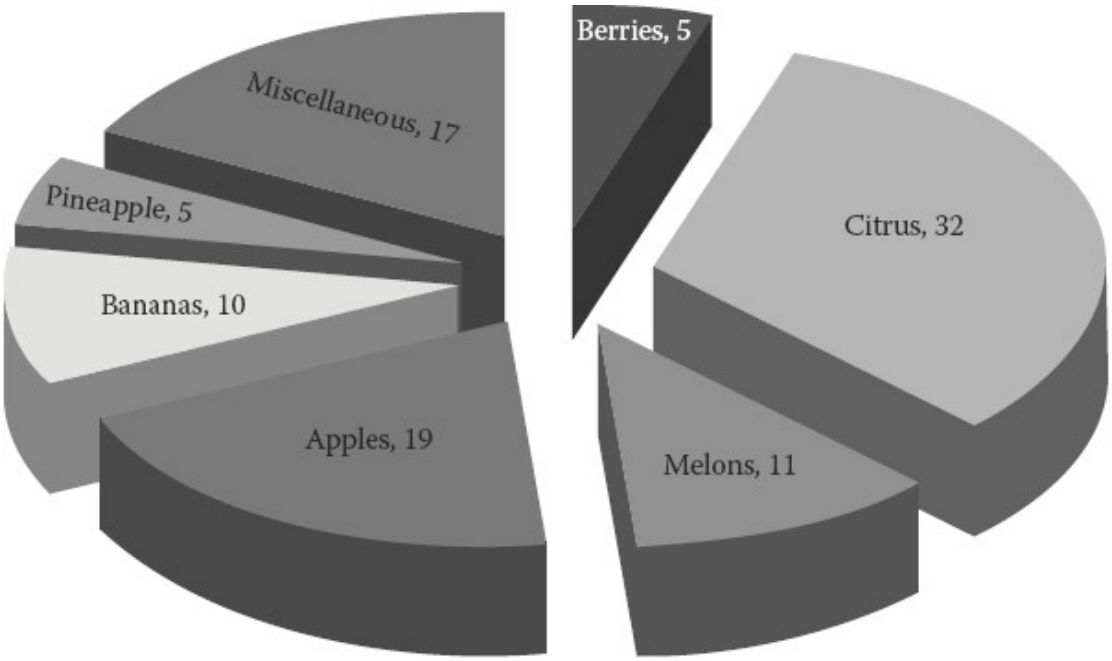
About 95% of the cranberries cultivated are processed into products such as juice drinks, sauce, and sweetened, dried cranberries. The remaining 5% are sold fresh to consumers. Cranberries used for processing are commonly frozen in bulk containers shortly after arriving at a receiving station. The primary method of harvesting cranberries takes advantage of the ability of cranberries to float. In this method, which is called wet harvesting, cranberry fields are flooded with water. After flooding, eggbeater-like devices stir up the water with sufficient force to dislodge the berries from the vines. When the berries float on the surface of water, they are pulled to the shore with hinged two-by-fours and loaded in trucks for delivery to the factory. Wet-harvested cranberries are generally processed for juice and sauce, because once a berry gets wet, there is increased chance of spoilage unless it is processed rapidly. Dry harvesting is the second method of harvesting cranberries. Because fully ripe berries are easily dislodged from their vines, this method employs a lawn mower-type machine that combs the berries from the vines. On arrival at the factory, dry-harvested cranberries are subjected to a unique test. The superior berries are sorted from those that are bruised, soft, or rotten by taking advantage of the fact that berries bounce when they are of good quality. Along a conveyor belt, each berry must successfully bounce over a series of wooden barriers. Berries that fail this simple test fall into a disposal bin. The ones that pass the bounce test are sold as fresh fruit, typically packaged in plastic (MotherLindas.com, http://www.motherlindas.com/cranberries.htm).

There are approximately 450 cranberries in 1 pound, 4,400 cranberries in 1 gallon of juice, and 45,000 cranberries in a 100-pound barrel. It takes about 200 cranberries to make one can of cranberry sauce (foodreference.com, http://www.foodreference.com/html/fcranberries.html). The cranberry is an underconsumed fruit in the United States. Consumption of different types of fruits in the United States is shown in Figure 6.1. Americans eat on an average 326 g of fruit per day, and only 5.3% of this intake is berries (18 g). This translates to 2.8 g or about three cranberries per day. This is equivalent to drinking 0.25 mL of cranberry juice per day. The dominant cranberry product consumed in the United States is juice. In comparison, the per capita fruit consumption in the European Union in 2007 was considerably less, only 266 g/day (Freshfel

Europe 2009). Average per capita consumption of berries in Norway was only 5 g/day (Ellingsen et al. 2008). Thus, Europeans on the average consume fewer berries than the U.S. population.

FIGURE 6.1. Per capita consumption of fruits in the United States in 2007.

FIGURE 6.1



Per capita consumption of fruits in the United States in 2007. (From USDA/Economic Research Service. Food Availability (Per Capita) Data System. http://www.ers.usda.gov/Data/FoodConsumption. Data last updated February 16, 2010. With permission.)

6.3 PHYTOCHEMICAL COMPOSITION

It is interesting to discover via a PubMed search that there are over 52,000 references for flavonoids (http://www.ncbi.nlm.nih.gov/sites/entrez, accessed Nov. 14, 2010). Among the subclasses, the order of references is flavonols > flavonones > anthocyanins > flavanols (catechins). Table 6.1 lists the potentially bioactive compounds identified and often quantified in cranberries. Table 6.2 lists the flavonoids quantified in cranberry as listed in the U.S. Department of Agriculture (USDA) database. Representative structures of the major constituents are shown in Figures 6.2 and 6.3.

TABLE 6.1. Potentially Bioactive compounds Present in Cranberries and Cranberry Juice.

TABLE 6.1

Class of Compounds	Citation and Derivatives Identified
Anthocyanins	Functional group attached; Cunningham et al. 2003; Wu and

Class of Compounds	Citation and Derivatives Identified
	Prior 2005
Cyanidin	Galactoside, arabinoside, and glucoside
Peonidin	Galactoside, arabinoside, glucoside, and digalactoside
Malvidin	Galactoside and arabinoside
Pelargonidin	Galactoside and arabinoside
Delphinidin	Arabinoside
Petunidin	Galactoside
Flavonols	Yan et al. 2002; Zheng and Wang 2003; Cunningham et al. 2003; Vvedenskaya et al. 2004
Quercetin	Galactoside (hyperin), rhamnoside (quercetin), xyloside
	(avicularin), and glucose (isoquercetin)
Kaempferol	Glucoside
Myrcetin	Xylopyranoside and arbinofuranoside
Catechins and Flavanols	Harnly et al. 2006
Epicatechin, catechin, epigallocatechin, epigallocatechin gallate, catechin gallate, and gallocatechin gallate *Proanthocyanidins (dimers, trimers, and oligomers*)	Prior et al. 2001; Gu et al. 2004
Procyanidin B2 EC-(4β→8)-EC Procyanidin A2 EC-(4β→8)-EC	
EC-(4β→6)-EC-(4β→8, 2β→*O* ↓ 7)-EC	
EC-(4β→8, 2β→O→7)-EC-(4β→8)-EC	
4–6mers	
7–10mers	
>10mers	
Benzoic and phenolic acids	Zuo, Wang, and Zhan 2002; Zheng and Wang 2003; Cunningham et al. 2003; Zhang and Zuo 2004; Marks, and Crozier 200
Benzoic acid; salicylic acid; *m*-hydroxybenzoic acid; *p*-hydroxybenzoic acid; 2,3-dihydroxybenzoic acid; 2,4-	Zafriri et al. 1989; Turner et al. 2005; Baur and Sinclair 2006; Turner et al. 2007 7

Class of Compounds	Citation and Derivatives Identified
dihydroxybenzoic acid; 3,4-dihydroxybenzoic acid; *p*-hydroxyphenylacetic acid; vanillic acid; *trans*-cinnamic acid; *o*-hydroxycinnamic acid; *p*-coumaric acid; *o*-phthalic acid; caffeic acid; ferulic acid; sinapic acid; chlorogenic acid; and 5-*O*-caffeoylquinic acid *Non avonoid polyphenols*	
Phloretin-2-glucoside (phloridzin), ellagic acid, 2-*O*-(3,4- diydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate, *cis*-resveratrol, *trans*-resveratrol, and secoisolariciresinol *Terpenes and sterols*	Jensen et al. 2002; Murphy et al. 2003; He and Liu Turner et al. 2007
Oleanolic acid, ursolic acid (UA), *cis*-3-*O*-*p*-hydroxy cinnamoyl UA, *cis*-3-*O*-*p*-hydroxy cinnamoyl UA, ß-sitosterol, ß-sitosterol-3-*O*-ß-D-glucoside, monotropein, 6,7-dihydro monotropein, 10-*p*-*cis*-coumaroyl-1S-dihydro monotropein, and 10-*p*-*trans*-coumaroyl-1S-dihydro monotropein	

Potentially Bioactive compounds Present in Cranberries and Cranberry Juice.

TABLE 6.2. Content of Flavonoids in Cranberry Fruit by Flavonoid Class, as Reported by the USDA Databases on Nutrient Composition of Foods.

TABLE 6.2

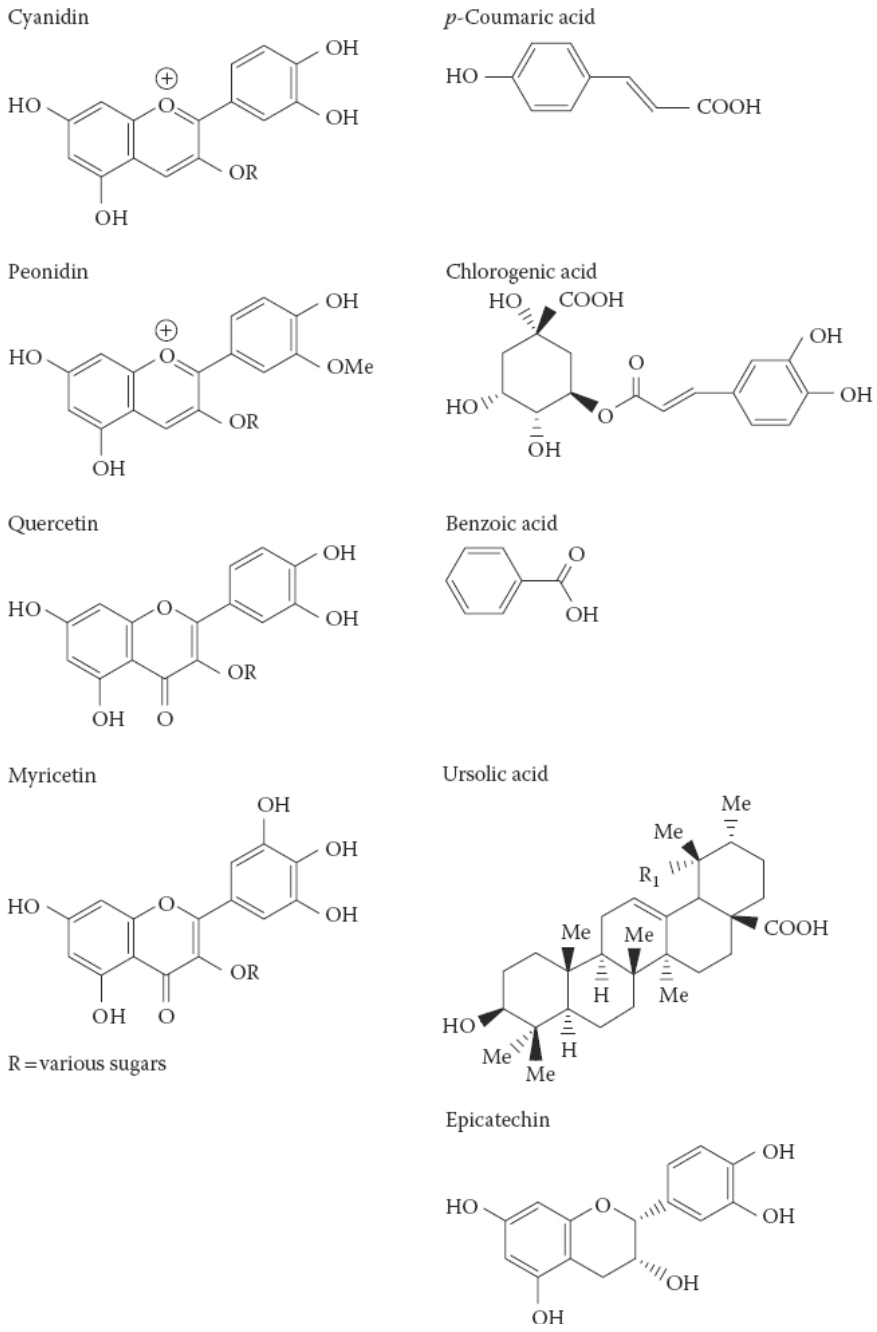
Flavonoid	Milligrams per 100 Gram of Whole Cranberry Fruit
Flavonols, total[c]	21.96
Quercetin[a]	15.09 ± 1.06
Myricetin[a]	6.78 ± 1.67
Kaempferol[a]	0.09 ± 0.03
Anthocyanins, total[c]	91.57
Cyanidin[a]	41.81 ± 2.86
Peonidin[a]	42.10 ± 3.64
Delphinidin[a]	7.66 ± 1.93

Flavonoid	Milligrams per 100 Gram of Whole Cranberry Fruit
Flavan-3-ol monomers[b]	7.26
(-)Epicatechin[a]	4.37 ± 0.93
(-)Epigallocatechin[a]	0.74 ± 0.28
(-)Epigallocatechin gallate[a]	0.97 ± 0.48
(+)-Catechin[a]	0.39 ± 0.16
PACs, total[c]	411.5
Dimers[b]	25.93 ± 6.12
Trimers[b]	18.93 ± 3.39
4–6mers[b]	70.27 ± 13.07
7–10mers[b]	62.90 ± 14.71
Polymers[b]	233.48 ± 49.08

Content of Flavonoids in Cranberry Fruit by Flavonoid Class, as Reported by the USDA Databases on Nutrient Composition of Foods.

FIGURE 6.2. Structures of some of the major anthocyanins (cyanidin and peonidin), flavonols (quercetin and myricetin), phenolic and organic acids (p-coumaric acid, chlorogenic acid, and benzoic acid), flavan-3-ols (epicatechin), and terpenoids (ursolic acid) in cranberry fruit.

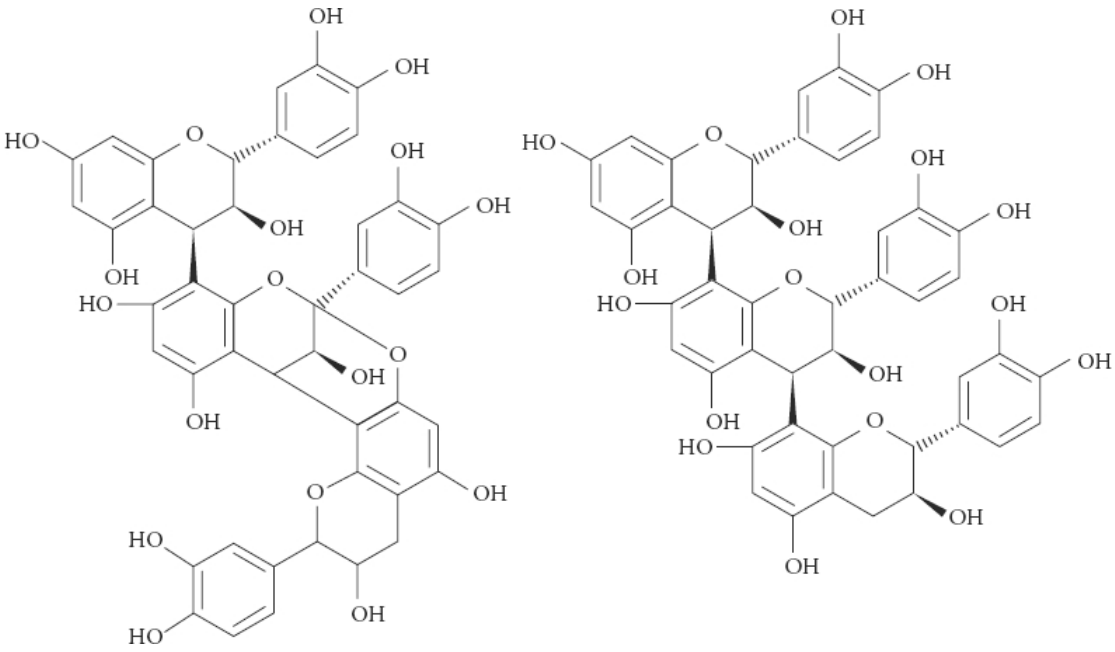
FIGURE 6.2



Structures of some of the major anthocyanins (cyanidin and peonidin), flavonols (quercetin and myricetin), phenolic and organic acids (p-coumaric acid, chlorogenic acid, and benzoic acid), flavan-3-ols (epicatechin), and terpenoids (ursolic acid) in cranberry (more...)

FIGURE 6.3. Cranberry uniquely contains A-type linkage (left), whereas other foods contain B-type linkage (right).

FIGURE 6.3



Cranberry uniquely contains A-type linkage (left), whereas other foods contain B-type linkage (right).

6.3.1 Anthocyanins

When one thinks of cranberries, it is the color red that comes to mind. It is due to the presence of anthocyanins, which have been extensively studied for health benefits. There are six aglycones in the anthocyanin class, and the number of potential isomers is extremely large due to different positions of sugar attachment and the large number of different mono- and disaccharides (glycosides) and, the less common, but possible, phenolic acids and acyl groups that can be attached. Anthocyanin content has been reported to be as high as 91.5 mg/100 g ripe fruit at harvest depending on the cultivar (Wang and Stretch 2001; Wu et al. 2006). Fruit of the early black cultivar tends to contain more anthocyanins and proanthocyanidins (PACs) than other cranberry cultivars (Wang and Stretch 2001; Vorsa and Howell 2003). Cranberry, compared to many other berries, has a very small number of anthocyanin isomers (n = 13), the major ones being galactosides and arabinosides of cyanidin and peonidin. A very similar anthocyanin profile exists in cranberry juice (Fuleki and Francis 1968; Ohnishi et al. 2006). Only the most recent references are listed in Table 6.1, since the newer technology liquid chromatography-mass spectrometry (LC-MS)/MS is better able to unequivocally identify compounds in mixtures than the classical methods of column chromatography followed by crystallization and spectral investigation. Whole cranberries contain a very broad concentration range of anthocyanins, 180–596 mg/kg fresh weight (fw; Bilyk and Sapers 1986), which also varies according to maturity from 0.8 to 111.0 mg/kg fw from the green to dark red stage (Celik et al. 2008). Cranberry juice contains much lower levels of anthocyanins, ranging from 1.3 mg/100 mL (Prior et al. 2001) to 2.5 mg/100 mL of juice (Cunningham et al. 2003).

6.3.2 Flavonols

The total amount of flavonols in cranberry ranges from 200 to 400 mg/kg (Zheng and Wang 2003; Cunningham et al. 2003; Vvedenskaya et al. 2004; Neto 2007b). Cranberry is the best source of flavonols among 30 flavonol-containing plant foods studied (Aherne and O'Brien 2002), and the flavonol content of cranberry is almost twice as high as 12 other commonly consumed fruit juices, including pomegranate and grape (Mullen, Marks, and Crozier 2007). Quercetin is the most abundant flavonol in cranberry, and it varies from 11 to 25 mg/100 g, primarily as the 3-o-galactoside (Yan et al. 2002; Vvedenskaya et al. 2004). Cranberry is also the best source of quercetin (Manach et al. 2004). Myricetin is the second most abundant flavonol, followed by kaempferol (Vvedenskaya et al. 2004). These compounds are yellow in color, and there are 20 different flavonol glycosides in cranberry, as confirmed by another article (Vvedenskaya and Vorsa 2004).

6.3.3 Flavan-3-ols (Catechins)

Total catechins in raw cranberry average 17 mg/100 g, with epicatechin being the most abundant (Harnly et al. 2006). Berries have the highest catechin content among fruits. Cranberries have the highest levels of monomers (catechin + epicatechin) among berries, and monomer levels in cranberries are twice as high as those in blueberries (Gu et al. 2004). Cranberry juice contains 6 mg/l of catechins (Gu et al. 2004). One liter of cranberry juice has catechin and epicatechin monomers as 100 g of dark chocolate.

6.3.4 Proanthocyanidins

These compounds are the largest class of potential bioactives in cranberries. The PACs are also commonly called condensed tannins or poly flavan-3-ols, and are responsible for the bitter astringent taste of cranberries due to the binding of saliva proteins. Cranberries have the most dimers, trimers, 4–6mers, and 7–10mers compared with any other fruit studied (Gu et al. 2004). The most unique aspect of cranberry polyphenols is the occurrence of A-type linkages (C2→O→C7) between epicatechin units, as shown in Figure 6.3 (Foo et al. 2000b). These linkages are found elsewhere only in peanuts, blueberries, and plums (Gu et al. 2003). The average concentration of PACs is 419 mg/100 g (0.4%) by weight in cranberries and 231 mg/l in cranberry juice (Gu et al. 2004). These compounds exhibit potent in vitro antiadhesion bioactivity and are present in much higher concentrations in cranberries than in other foods.

6.3.5 Benzoic And Phenolic Acids

Benzoic and phenolic acids represent 0.57% of the weight of fresh cranberries (Zuo, Wang, and Zhan 2002). Benzoic acid forms 80% of the total organic acids contained in cranberry juice. There exist 14 other benzoic and phenolic acids in cranberry juice, and p-coumaric acid is the most prevalent hydroxycinnamic acid. Many of these acids are bound to glucose and polysaccharides in cranberry (He and Liu 2006).

6.3.6 Nonflavonoid Polyphenols

Phloridzin is the most prevalent member of the class of dihydrochalcones present in cranberries at 120 mg/kg (Turner et al. 2007). This compound is present in very high quantities in apple, and is a dihydrochalcone with glucose-lowering activity in an animal

model of diabetes (Masumoto et al. 2009). Resveratrols, one of the most studied polyphenolic compounds, with 2937 PubMed references, are found in cranberry juice at extremely low levels of 0.2 mg/l (Wang et al. 2002). This is much lower than the resveratrol content of red wine (14 mg/l; Baur and Sinclair 2006). Other compounds belonging to this stilbene class are present at very low levels in cranberry juice, and thus are probably nonbioactive.

6.3.7 Terpenes And Sterols

Terpenes include the volatile compounds that are responsible for the flavor and aroma of cranberries (Croteau and Fagerson 1968), as well as larger oily or waxy compounds. Cranberry fruit contains the triterpenoid ursolic acid (UA) in its peel (Figure 6.3), existing in aglycone form as well as in cis and trans p-hydroxycinnamate esters (Murphy et al. 2003). Quantitative analysis of cranberry fruit and products by LC-MS has determined the UA content of the whole cranberry fruit of different cultivars to be between 60 and 110 mg per 100 g of fresh fruit (Kondo 2006). UA has been reported to be a constituent of other fruits, including apples and highbush blueberries (Wang et al. 2000). The iridoid glycosides listed in Table 6.1 are believed to be unique to the Vaccinium species. Cranberries also contain the carotenoid lutein, as well as other carotenoids in lesser quantities. It is worth noting that although the compounds listed in Table 6.2 are the result of studies conducted on the American cranberry (V. macrocarpon), a similar variety of anthocyanins, flavonols, nonflavonoid polyphenols, and phenolic acids are found in the European cranberry (V. oxycoccus) even though this variety has not been studied as extensively as its. American counterpart (Häkkinen et al. 1999; KähkÖnen, Hopia, and Heinonen 2001).

6.4 BIOAVAILABILITY OF CRANBERRY PHYTOCHEMICALS

The first evidence of human absorption of cranberry compounds was demonstrated by a single subject who consumed 1800 mL of 27% commercial cranberry juice after an overnight fast (Zhang and Zuo 2004). The plasma was then extracted and derivatized prior to assay. Sixteen compounds were identified in plasma by GC-MS from blood samples drawn 45 and 270 minutes after consumption. After 45 minutes, five acids were identified: (1) benzoic acid, (2) o-hydroxybenzoic acid (salicylic acid), (3) p-hydroxyphenylacetic acid, (4) 2,3-dihydroxybenzoic acid, and (5) 2,4-dihydroxybenzoic acid. Phenylacetic acid and dihydroxybenzoic acid are not found in cranberry juice and, thus, they are the products of polyphenol breakdown or bacterial metabolism in the gut. At 270 minutes, the same five acids and ferulic acid plus sinapic acid were found. Anthocyanins were not analyzed in the study. In 2005, an early-morning urinary excretion study was conducted with 11 females who were administered a commercial cranberry juice three times a day (250 mL each) for 2 weeks (Duthie et al. 2005). High-performance liquid chromatography (HPLC) with electrochemical detection was used after urine hydrolysis with NaOH and extraction. After 1–2 weeks, the urinary level of salicyluric acid (metabolite of salicylic acid) significantly increased. This compound was also increased in the fasting plasma after 2 weeks of consumption of cranberry juice. A Japanese group gave 200 mL of 100% cranberry juice to 11 subjects, and urine was collected before administering the juice and again 24 hours afterwards (Ohnishi et al. 2006). Six of the 12 anthocyanin glycosides identified in cranberry juice by LC-MS/MS

were found in the collected urine. Peonidin 3-o-galactoside, the second most plentiful anthocyanin in the juice, was the major anthocyanin in the urine. Over 5% of the consumed anthocyanins were found in the urine. This level of absorption is at least 10 times higher than that found for other berry juices, which ranges from 0.08% for blood orange juice (Giordano et al. 2007) to 0.2% for red wine and red grape juice (Frank et al. 2003) and 0.4% for black currant juice (Bitsch et al. 2004). A safety study in which a cranberry powder was given to 65 healthy women for 8 weeks at a dose of 1200 mg/day and the effects were compared with a placebo (Valentova et al. 2007) showed no toxicity for the powder. In decreasing order of content, hippuric acid, salicyluric acid, quercetin glucuronide, and dihydroxybenzoic acid isomers were found in the urine by LC-MS.

6.5 HUMAN STUDIES RELEVANT TO HEART DISEASE AND DIABETES

Growing evidence points to many potential pharmacological activities for cranberry and cranberry juice. Due to its high content of flavonoids and phenolic acids, cranberry ranks highly among fruits in both antioxidant quality and quantity (Vinson et al. 2001). In fact, cranberry has the highest level of fw polyphenols among a group of 22 fruits studied. These antioxidant properties are likely to contribute to cranberry's disease-fighting ability.

Cranberry compounds can cause an improvement in antioxidant status, which might be beneficial with respect to chronic diseases. The potential cardiovascular benefits have been reviewed by two groups of researchers (McKay and Blumberg 2007; Ruel and Couillard 2007). A single dose of 500 mL 27% cranberry administered to nine healthy women increased plasma antioxidant capacity against a sucrose control (Pedersen et al. 2000). The authors stated that this increase was due to the presence of ascorbic acid (vitamin C) in cranberry juice. Unfortunately, ascorbic acid was not included in the control sucrose solution. Another group found that cranberry juice relative to a control containing sugars and ascorbic acid significantly raised the plasma antioxidant capacity in normal subjects (Vinson et al. 2008). They also found that when cranberry juice was spiked on human plasma, the lipoproteins low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were protected from oxidation in a dose-dependent manner. This second effect is directly related to atherosclerosis (Steinberg 2009).

The proof of benefit for disease prevention should be shown by a human supplementation study. There have been a few of these studies for cranberry, all done in the last 6 years. Cranberry powder equivalent to 240 mL of cranberry juice per day was given to 12 diabetic men and 15 diabetic women for 12 weeks (Chambers and Camire 2003). There was no effect on glucose control. According to the authors, no change was observed on glucose control because the basic cranberry powder might have undergone processing in its production from cranberry juice. In 2008, a Japanese group reported the effect of a cranberry powder and a placebo given to 30 type 2 diabetic subjects for 12 weeks (Lee et al. 2008). There was no effect of cranberry on fasting glucose or glycated hemoglobin levels. However, it was found that total cholesterol and LDL significantly decreased and the cholesterol/high-density lipoprotein (HDL) ratio significantly increased in the cranberry group. All the subjects were taking oral hypoglycemic drugs and, thus, their plasma glucose levels were under control. In

another study, type 2 diabetics (n = 12) were given a low-calorie cranberry juice at a dose of 240 mL (Wilson et al. 2008). The juice was found to give a more favorable glucose and insulin response than a sugared water control.

Cranberry juice was given to 20 women at a dose of 750 mL/day for 2 weeks (Duthie et al. 2006). No effect on plasma lipids, antioxidant enzyme, or DNA oxidation was found, but plasma ascorbic acid was increased. Healthy men (n = 21) were given 7 mL/kg of cranberry juice for 14 days (Ruel et al. 2005). Although there was no effect on lipids, oxidized LDL was significantly decreased (10%) and plasma antioxidant capacity was significantly increased (7%) in the subjects. Inflammatory markers were not improved. In the latest study by the same group, men with elevated LDL and body mass index (BMI) were given low-calorie cranberry juice at doses of 125, 250, and 500 mL/day for three successive 4-week periods (Ruel et al. 2007). Significant decreases in oxidized LDL, systolic pressure, and the inflammatory markers ICAM-1 and VCAM-1 were recorded at the end of the study. All cranberry effects in this study were found to be beneficial with respect to heart disease.

6.6 INVESTIGATION OF CRANBERRY CONSTITUENTS THAT CONTRIBUTE TO IN VITRO ANTICANCER ACTIVITY

Cranberry's role as a potential chemopreventive agent is gradually emerging from in vitro model studies by various researchers. Cranberry compounds may act against cancers by inhibiting oxidative stress or by other pathways. Over the past 10 years, numerous in vitro studies have appeared addressing the effects of cranberry and its constituents against tumor cell proliferation and the possible mechanisms of action. Several reports of in vivo studies have appeared recently, lending support to the tumor-inhibiting potential of cranberry. These studies are discussed. A summary of in vitro and in vivo studies on the anticancer properties of cranberry, the constituents contributing to these properties, and some possible mechanisms of action are discussed.

The first in vitro report of anticancer activity in Vaccinium fruit appeared in 1996. A University of Illinois study reported that extracts of cranberry, bilberry, and other species inhibit ornithine decarboxylase (ODC) expression and induce the xenobiotic detoxification enzyme quinone reductase in vitro (Bomser et al. 1996). Some Canadian researchers reported that cranberry juice extracts inhibit breast tumor cell growth (Guthrie 2003), and later showed that an extract of cranberry press cake inhibits proliferation of MCF-7 and MDA-MB-435 breast cancer cells (Ferguson et al. 2004). Later studies by various researchers have focused on identifying the anticancer constituents of cranberry, which fall within several possible classes, including triterpenoids and flavonoids.

6.6.1 Ursolic Acid And Derivatives

A bioassay-guided fractionation approach was used to examine in vitro the antitumor activities of whole cranberry fruit and juice, extracts and fractions, and finally individual compounds or subfractions within structural classes. An ethyl-acetate extract of whole cranberry fruit inhibited growth of human tumor cell lines in vitro (Yan et al. 2002). From ethyl-acetate-soluble extracts, two hydroxycinnamate esters of UA were isolated and identified that inhibited the growth of several types of human tumor cells in vitro, including

MCF-7 breast, HT-29 colon, DU145 prostate, H460 lung, ME180 cervical epidermoid, and K562 leukemia cell lines (Murphy et al. 2003). Growth-inhibitory concentrations (GI50) for esters were 11–28 µg/mL, depending on the cell line. LC-MS analysis of cranberry fruit found that in addition to UA, its hydroxycinnamate esters are present in whole cranberry fruit in quantities averaging about 15–20 mg per 100 g of fresh fruit (Kondo 2006). UA isolated from cranberry fruit was also reported to inhibit the proliferation of HepG2 human liver cancer cells and MCF-7 cells (He and Liu 2006). It was also found to inhibit tumor colony formation in a dose-dependent manner in HT-29 and HCT116 colon tumor models over a 2-week period, based on clonogenic assays (Liberty, Hart, and Neto 2007).

UA has been reported as a constituent of herbal medicines marketed worldwide for treating inflammatory conditions (Kim et al. 2004), but bioavailability data on this compound is lacking. In vivo cancer studies of UA are scarce, but one mouse model study reported that a UA dose of 100 mg/ kg inhibits murine fibrosarcoma FSaII growth (Lee et al. 2001). Numerous reports have appeared on the in vitro antitumor activity of UA (Novotny, Vachalkova, and Biggs 2001), and these suggest a variety of mechanisms of action, including early G1 cytostatic effect (Es-Saady et al. 1996), induction of apoptosis (Baek et al. 1997), enhancement of intracellular Ca2+ signaling (Novotny, Vachalkova, and Biggs 2001), enhanced release of cytochrome c, caspase activation (Andersson et al. 2003) and downregulation of inhibitor of apoptosis proteins (c-IAPs; Choi et al. 2000), increased expression of p21WAF1 (Kim et al. 2000), and decreased matrix metalloproteinase (MMP)-9 expression (Cha et al. 1996). In addition to inhibiting the growth of several tumor cell lines, the hydroxycinnamoyl esters of UA were found to strongly inhibit expression of both MMP-2 and MMP-9 at micromolar concentrations in a DU-145 prostate tumor model (Kondo et al. 2004).

6.6.2 Polyphenolics

Polyphenolic compounds are expected to play a key role in chemoprevention; thus, many in vitro cranberry studies focus on the berry's polyphenolic constituents. Cranberry extracts containing PACs and other flavonoids were reported to inhibit ODC activity linked with cell proliferation in mouse epithelial (ME-308) cells (Kandil et al. 2002). Characterization of an active subfraction revealed the presence of dimers and oligomers of catechin/epicatechin, monomeric catechins, and quercetin glycosides. Water-soluble cranberry polyphenolic extracts from commercial cranberry powder were found to inhibit proliferation of several human tumor cell lines (Seeram et al. 2004) including two oral (CAL27 and KB), four colon (HT-29, HCT-116, SW480, and SW620), and three prostate (RWPE-1, RWPE-2, and 22Rv1) cancer cell lines. Although both anthocyanin and PAC subfractions inhibited proliferation, the total polyphenolic extract was found to be more effective in this study. The primary polyphenolic constituents of cranberry are flavonols, anthocyanins, and PACs. These will be considered in Sections 6.6.3 through 6.6.5.

6.6.3 Quercetin

Quercetin, a constituent of many fruits and vegetables, is widely reported to have antiproliferative and antineoplastic activities in vitro against a variety of cell lines (Ranelletti, Maggiano, and Serra 2000; Morrow et al. 2001; Sesink, O'Leary, and Hollman 2001; Lee,

Huang, and Hwang 2002; Harris et al. 2005; Richter et al. 1999; Volate et al. 2005). Its mechanisms of action include induction of apoptosis, with cell-cycle arrest in G1 phase (Richter, Ebermann, and Marian 1999; Choi et al. 2001); inhibition of epidermal growth factor (EGF) receptor expression and associated tyrosine kinase activity (Richter, Ebermann, and Marian 1999; Lee, Huang, and Hwang 2002); reduction of Ras protein expression (Ranelletti, Maggiano, and Serra 2000); increased expression of endogenous inhibitors of MMPs (Morrow et al. 2001); and phytoestrogenic interaction with estrogen receptors α and β (ERα and EBβ of human mammary MCF-7 cells; Harris et al. 2005). Quercetin is a constituent of an ethyl-acetate-soluble extract of cranberry fruit that inhibits tumor cell growth; it was nearly as effective as UA esters against the growth of MCF-7 breast adenocarcinoma, HT-29 colon adenocarcinoma, and K562 leukemia cell lines (Murphy et al. 2003). In a separate study, it was found to inhibit proliferation of HepG2 liver cancer cells (He and Liu 2006). Given the known properties of quercetin, it is a likely contributor to the observed in vitro anticancer activity of whole cranberry extracts. In vivo, quercetin glycosides are usually metabolized to sulfates or glucuronides (Sesink, O'Leary, and Hollman 2001). In a colon cancer study, a quercetin-enriched diet decreased formation of cancer precursor aberrant crypt foci fourfold in mice, and the evidence suggest that quercetin acted through induction of apoptosis via a mitochondrial pathway involving modulation of Bax and Bcl-2 protein expression (Volate et al. 2005).

6.6.4 Anthocyanins

As powerful antioxidants, anthocyanins may be expected to inhibit oxidative processes linked with tumorigenesis. In vitro bioassays with cranberry anthocyanins show little direct antiproliferative or growth-inhibitory properties. Purified cyanidin-3-galactoside was evaluated in eight tumor cell lines in vitro using the sulforhodamine (SRB) assay and the highest concentration tested (250 μg/mL) showed less than 50% growth inhibition (Murphy et al. 2003). In another study, an anthocyanin subfraction from cranberry was found to limit growth in three prostate tumor lines (RWPE-1, RWPE-2, and 22Rv1) by 50–70%, but it did not significantly inhibit oral or colon tumor cell line proliferation (Seeram et al. 2004). Anthocyanins, including those from cranberry, have been implicated in the observed antiangiogenic properties of mixed berry extracts (Roy et al. 2002; Bagchi et al. 2004). Anthocyanin-rich extracts from a mixture of berry fruits were reported to inhibit tumor necrosis factor α (TNF-α)-induced vascular endothelial growth factor (VEGF) expression and to decrease hemangioma formation and tumor growth (Atalay et al. 2003). Anthocyanins, though not especially cytotoxic, may nonetheless play a role in limiting carcinogenesis by inhibiting other activities related to tumor formation.

6.6.5 Anticancer Activities Of Cranberry Proanthocyanidins

Many studies report cranberry PACs are major contributors to anticancer activity (Kandil et al. 2002; Ferguson et al. 2004; Seeram et al. 2004). In fractionation studies of whole fruit, a PAC fraction selectively inhibited the proliferation of H460 human large cell lung carcinoma, HT-29 colon adenocarcinoma, and K562 chronic myelogenous leukemia cells in vitro. A subfraction retaining the activity in those three cell lines was isolated and characterized by matrix-assisted laser desorption/ ionization (MALDI)-TOF (time-of-flight)-MS, and was found

to contain PAC oligomers composed primarily of four to seven epicatechin units with at least one or two A-type linkages between the units (Neto et al. 2006). In clonogenic assays for tumor colony formation with HT-29 and HCT-116 colon tumor cell lines, a dose-dependent decrease in the number and size of tumor colonies was observed when cells were treated with a PAC fraction prepared from the early black variety of cranberry fruit (Liberty et al. 2009). The MALDI-TOF-MS characterization of this PAC fraction revealed that it was composed primarily of trimers through hexamers of epicatechin with both A- and B-type linkages. The fraction inhibited growth more effectively than the whole polyphenolic extract, with over 50% inhibition of colony formation in HCT116 observed at concentrations less than 10 μg/mL (Liberty, Hart, and Neto 2007).

The structures of PACs in cranberry fruit and cranberry products are complex, and further investigation is needed to determine any link between structure and activity of PAC. The MALDI-TOF-MS analysis found that PAC oligomer fractions from whole cranberry contained molecules up to 12 degrees of polymerization (DP) in size with as many as four A-type linkages. Although most fractions contained epicatechin units exclusively, some epigallocatechin unit masses were also detected (Neto et al. 2006). The effect of A-type linkages and oligomer size on the antitumor activity of PACs is the subject of ongoing studies. Earlier reports on PACs from various sources suggest that A-type linkages influence tumor-inhibitory and tumor-selectivity properties. A screening of small polyflavan-3-ols from different plants against GLC4 lung and COLO 320 colon carcinomas found that a trimer with an A-type linkage was more cytotoxic than dimers with A-type linkages and trimers with only B-type linkages (Kolodziej et al. 1995). In another study, wild blueberry PACs containing A-type linkages also showed selectivity, with a greater growth-inhibitory effect occurring with androgen-sensitive LNCaP prostate cancer cells than with androgen-insensitive DU145 cells (Schmidt, Erdman, and Lila 2006).

A recent study on cranberry PACs reports some promise for cranberry in alleviating cancer of the esophagus. A known risk factor for this type of cancer is acid reflux (Herulf et al. 1999). An in vitro model study employing SEG-1 human esophageal adenocarcinoma was used to investigate cranberry's effects on acid-induced cell proliferation. Cells were pretreated with PAC extract at concentrations of 25 or 50 μg/mL and then pulsed with acidified medium to simulate reflux. Maximum proliferation was induced 6 hours after treatment; further, proliferation was inhibited significantly in the PAC-treated cells. Annexin-V staining showed that apoptosis had occurred in treated cells, and flow cytometry experiments found these cells arrested at the G1 checkpoint (Kresty, Howell, and Baird 2008).

Recent findings suggest that cranberry PACs may work together with platinum drugs to limit the proliferation of ovarian cancer cells. In a study using a platinum-resistant SKOV-3 human ovarian adenocarcinoma cell line, treatment with a sublethal concentration of paraplatin together with an isolated cranberry PAC fraction was found to improve efficacy in decreasing cell proliferation as compared to treatment with PACs alone (Singh et al. 2009).

6.7 POSSIBLE CHEMOPREVENTIVE MECHANISMS AND EFFECTS

Berry bioactives may act individually, additively, or synergistically to prevent cancer proliferation (Seeram 2006). Inhibition of tumorigenesis by cranberry can involve complementary or synergistic activities between the flavonols, PACs, UA, and anthocyanins, since all these compounds have been reported as showing antiproliferative properties. Possible mechanisms of action for chemoprevention supported by in vitro evidence include induction of apoptosis, decreased invasion of surrounding tissue due to MMP inhibition, reduction of ODC expression and activity, and inhibition of oxidative or inflammatory processes.

6.7.1 Apoptosis

Induction of apoptosis is thought to play a role in the tumor-inhibitory activity of dietary phytochemicals including resveratrol (Joe et al. 2002) and epigallocatechin gallate (Yang et al. 1998). Recent reports suggest that apoptosis may play a key role in cranberry's ability to limit tumor cell growth. This activity may be associated with the presence of quercetin, UA, and/or PACs, which are all known to induce apoptosis (Baek et al. 1997; Choi et al. 2001). Dose-dependent induction of apoptosis by cranberry was observed in breast tumor models. An antiproliferative fraction from cranberry press cake also induced apoptosis in MDA-MB-435 breast tumor cells as determined by Annexin-V staining (Ferguson et al. 2004). An 80% aqueous acetone extract of whole cranberry fruit was reported to increase apoptosis in MCF-7 cells by 25% (Sun and Liu 2006), although at a concentration (50 mg/mL) much higher than would likely be encountered in vivo.

The whole polyphenolic extract of cranberry fruit was observed to increase apoptosis substantially in tumorigenic (MCF-7) breast cells as compared to nontumorigenic (MCF-10A) breast cells. At 250 µg/mL, the cranberry extract increased the baseline apoptosis rate to 92% in MCF-7 cells, without increasing apoptosis in MCF10A cells to a significant extent (Griffin et al. 2005). In colon tumor cell lines HCT116 and HT-29, treatment with UA or with cranberry PACs was found to induce significant rates of apoptosis at concentrations below 100 µg/mL (Liberty, Hart, and Neto 2007). Recent reports of the effects of cranberry PACs on human esophageal adenocarcinoma cells show that PACs induce apoptosis in this cell line (Kresty, Howell, and Baird 2008). Induction of apoptosis in SGC-7901 gastric cancer cells by an aqueous acetone extract of whole cranberry fruit was also reported (Liu et al. 2009). In a study of oral squamous cell carcinomas, a commercial cranberry powder reduced cell proliferation in SCC25 and CAL27 cell lines, and an upregulation of caspase-2 and caspase-8 messenger ribonucleic acid (mRNA) expression indicated that apoptosis played a role in reducing proliferation (Chatelain et al. 2008).

6.7.2 Matrix Metalloproteinase Inhibition

Phytochemicals from whole cranberry fruit may act against cancers by limiting the processes involved in tumor invasion and metastasis, particularly the expression of MMPs involved in remodeling the extracellular matrix (Pupa et al. 2002). Both the whole cranberry polyphenolic extract and a cranberry PAC fraction were found to inhibit the expression of MMP-2 and MMP-9 in the DU145 prostate tumor cell line in a dose-dependent manner (Neto et al. 2006). Similar activity was reported for a flavonoid-rich extract of cranberry's close relative, the highbush blueberry (V. angustifolium; Matchett et al. 2006).

Hydroxycinnamate esters of UA isolated from whole cranberry fruit were strong inhibitors of MMP-2 and MMP-9 protein expression, inhibiting expression significantly at concentrations of 10 µM or less (Kondo et al. 2004). This finding was consistent with the observed ability of UA to inhibit MMP expression in fibrosarcoma cells (Cha et al. 1996). Further studies are needed to determine the efficacy of cranberry and its PACs against tumor metastasis. Oligomers from grape seed have been found to possess antimetastasis activity both in vitro and in vivo (Mantena, Baliga, and Katiyar 2006).

6.7.3 Modulation Of Ornithine Decarboxylase

The biosynthesis and metabolism of polyamines (spermidine and spermine) involved in cell proliferation is controlled by enzymes such as ODC and spermidine/spermine N1-acetyltransferase, and ODC can be affected by dietary polyphenolics (Singletary and Meline 2001). Overexpression of these enzymes is observed in models of cancer where ODC can play a regulatory role in transformation, invasion, and angiogenesis (Auvinen 1997), and can be induced by proinflammatory agents such as lipopolysaccharides (LPSs) or tumor-promoters such as 12-o-tetradecanoyl phorbol-13-acetate (TPA). A cranberry fruit flavonoid fraction inhibits the activity of ODC in an ME-308 cell line, as determined by an assay measuring the conversion of substrate (Kandil et al. 2002). Cranberry was also found to influence the expression of ODC induced by LPS in an H-ras transformed mouse fibroblast model (Matchett et al. 2005). A whole cranberry polyphenolic extract produced a dose-dependent inhibition of LPS-induced ODC expression, and induction by LPS was abolished at extract concentrations of 100 µg/mL or less (Matchett et al. 2005).

6.7.4 Helicobacter pylori Inhibition

H. pylori infection is positively associated with the incidence of gastric cancer (Uemura et al. 2001); thus, the prevention of this infection may reduce cancer risk. Antibacterial adhesion studies demonstrate that in addition to inhibiting Escherichia coli adhesion, cranberry components inhibit adhesion of H. pylori to human gastric mucus (Burger et al. 2000). A randomized, double-blind, placebo-controlled trial provided some clinical support for this finding, with significantly lower levels of H. pylori infection observed in adults consuming cranberry juice (Zhang et al. 2005). The cranberry polyphenolic extract and other polyphenol-rich juices had a bacteriostatic effect on the growth of H. pylori in vitro, with morphological changes in bacteria seen at concentrations of 1 mg/mL (Matsushima et al. 2008).

6.7.5 Evidence From Animal Models

Tumor growth inhibition by cranberry in an animal model was first reported in 2006 (Ferguson et al. 2006) by a group of Canadian researchers. In this study, explants of U87 glioblastoma, HT-29 colon carcinoma, or DU145 prostate carcinoma were established in female Balb/c mice. Mice in the treatment groups were intraperitoneally injected with either a flavonoid-rich aqueous extract from cranberry press cake or a PAC fraction prepared from the whole fruit extract. Dosages of 100 mg/kg body weight PAC fraction or 250 mg/kg press cake extract were administered 10 times over a period of 24 days. Both treatments resulted in up to 40% reduction in the time required for U87 glioblastoma tumors to reach milestone

sizes. Flow cytometry experiments showed that the extracts arrest U87 cells in G1 phase after 24 hours, reducing the number of cells continuing to S phase. In mice given HT-29 tumor explants, the PAC treatment group exhibited significantly reduced tumor volume over the first 40 days compared to control. Proanthocyanidin treatment was also found to slow the growth of tumors in the DU145 group and induce complete regression of these tumors in the two treatment groups of mice (Ferguson et al. 2006).

Several animal studies have since appeared that examine the effects of cranberry treatment on models of cancer. A 2008 study used immune-competent syngeneic mice to investigate the effects of a nondialyzable (NDM) high-molecular-weight fraction from cranberry juice on the development of lymphoma (Hochman et al. 2008). The fraction was presumed to contain polyphenolic oligomers in the 12–30K weight range; therefore, exact structures could not be determined. Balb/c female mice were inoculated with Rev-2-T-6 lymphoma cells. The cranberry NDM fraction was injected into mice at nontoxic doses (2 or 4 mg) every 2 days for 2 weeks. At the end of the experiment, cranberry-treated mice showed no tumor development in comparison with the control group, in which 80% developed tumors within 3 weeks. The treated mice also showed an increase in the production of antibodies against lymphoma cells. In another study, Balb/c nu/nu mice were injected with human gastric cancer cell line SGC-7901 that had been pretreated with an acetone-soluble cranberry extract at doses ranging from 5 to 40 mg. After 4 weeks, the control group developed tumors (xenografts), whereas no tumors were observed in the highest cranberry dosage group and tumor size was reduced in mice receiving cranberry extract doses as low as 10 mg (Liu et al. 2009).

In a bladder cancer study that used Fischer-344 rats as a model, rats were treated with N-butyl-N(4-hydroxybutylnitrosamine) to induce cancer. Rats received cranberry juice concentrate via gavage at doses of 1.0 or 0.5 mL/rat/day (nontoxic doses) for up to 6 months. Both treatment groups showed a significant decrease in the number of bladder lesions, particularly papillomas, and the higher dosage group showed a significant decrease in number of carcinomas and a 31% decrease in the total weight of bladder lesions (Prasain et al. 2008). Quercetin, a key constituent of cranberry juice, was not detected in the plasma; however, both quercetin and its metabolite methyl quercetin were detected in the urines of the treated mice.

6.7.6 Anti-Lnflammatory Activities

The anti-inflammatory properties of phytochemicals may have an impact on many diseases including certain cancers. Cyclooxygenase (COX) is a key enzyme in the biosynthetic pathway to prostaglandins, which have many physiological roles, including the production of an inflammatory response. The COX-1 isozyme is expressed constitutively in all cells, whereas expression of COX-2 can be induced in response to inflammatory stimuli. The COX-2 is highly expressed in tumor tissues (Bottone et al. 2004), and studies show that nonsteroidal anti-inflammatory drugs (NSAIDs), for example, Sulindac, have a chemopreventive effect against colon cancer in cellular and animal models (Sheng et al. 1997; Fournier and Gordon 2000; Bottone et al. 2004). As COX-2 overexpression is thought to play a role in promoting certain cancers, inhibition of COX-2 activity or expression presents another potential route to

chemoprevention. Several individual constituents of cranberry may have anti-inflammatory properties. Inhibition of COX activity by anthocyanins, including those found in cranberry, was reported (Seeram et al. 2001). Pure cyanidin is an effective COX-2 inhibitor, which reduces activity by 47%, with activity superior to other anthocyanins or catechins (Seeram, Zhang, and Nair 2003). The observed COX-1 inhibition may also be relevant to cancer, since some evidence suggests that COX-1 specific inhibitors are as effective as COX-2 specific inhibitors in decreasing events related to tumor development (Bottone et al. 2004).

The question of whether cranberry can decrease the expression of COX-1 or COX-2 in cellular models remains to be answered; to date, no published studies are available that evaluate the effects of cranberry on COX expression in cancer models. Inhibition of COX-2 expression, if observed, could contribute to anticancer activity. Considering the known effects of compounds found in cranberry fruit, modulation of COX-2 expression and associated pathways is likely to be beneficial. Both UA and quercetin are established inhibitors of cellular COX expression (Subbaramaiah et al. 2000; O'Leary et al. 2004). The anti-inflammatory actions of triterpenes including UA have been reviewed (Safayhi and Sailer 1997), and studies support an anti-inflammatory role both in vitro (Ringbom et al. 1998; Subbaramaiah et al. 2000) and in vivo (Recio et al. 1995; Baricevic et al. 2001). UA inhibited COX-2 transcription in a human mammary oncogenic epithelial cell line (184B5/HER) by a mechanism believed to involve the protein kinase C signal transduction pathway (Subbaramaiah et al. 2000). Several anti-inflammatory activities of quercetin have also been reported. Quercetin reduced COX-2 mRNA expression in Caco-2 colon cancer cells, and both quercetin and its metabolite quercetin-3'-sulfate inhibited COX-2 activity (O'Leary et al. 2004). In a rat model of colitis, quercetin delivered in the form of rutin inhibited the TNF-α-dependent activation of nuclear factor κB (NF-κB), a transcription factor involved in the control of cell proliferation and inflammation, in a dose-dependent manner (Kim et al. 2005). Similarly, UA has been reported to suppress NF-κB activation (Shishodia et al. 2003). Quercetin has been reported to inhibit expression of inducible nitric oxide synthase, a promoter of inflammation that has also been linked to tumor angiogenesis, in cellular models (García-Mediavilla et al. 2007).

6.8 CRANBERRIES AND URINARY HEALTH

The use of cranberry juice to prevent UTIs has a long history that was for many years supported mainly by anecdotal evidence. This is no longer the case: A body of scientific evidence has accumulated to support the use of cranberry in the maintenance of urinary tract health. Studies started appearing in the 1980s demonstrating the ability of cranberry juice to prevent adherence of E. coli bacteria to uroepithelial cells and other eukaryotic cells (Sobota 1984; Zafriri et al. 1989; Ofek et al. 1991). As type 1-fimbriated bacteria were susceptible to the fructose in citrus fruit juices as well, the effect on type P-fimbriated E. coli was observed to be specific to cranberry (Zafriri et al. 1989) and other Vaccinium. During the mid-1990s, a clinical study conducted by Avorn et al. (1994) on the female residents of a long-term care facility found a significant decrease in bacteria in the urine after 1 month of cranberry juice consumption. Since then, at least 15 clinical trials have evaluated the

prophylactic effects of cranberry against urinary infections in a variety of populations. These studies are the subject of several detailed review articles (Howell 2002; Jepson and Craig 2007; Guay 2009).

For many years, scientists and health practitioners believed that the antibacterial effects of cranberry juice were due to acidification of urine by hippuric acid produced by the metabolism of the quinic acid in cranberries (Blatherwick 1914; Bodel, Cotran, and Kass 1959). However, this claim was never substantiated. Studies correlating urinary pH with cranberry juice consumption show either no significant change in urine acidity or only a slight reduction in pH, which is insufficient to cause a bacteriostatic effect (Howell 2002). Researchers began to examine other possible mechanisms of action, which led to the discovery of bacterial antiadhesion properties (Sobota 1984). Studies on antiadherence of uropathogenic P-fimbriated E. coli (UPEC) responsible for the majority of UTIs found that a high-molecular-weight NDM from cranberry inhibited adhesion (Ofek et al. 1991). Bioassay-guided fractionation of cranberry targeting the active compounds found that cranberry tannins (Howell et al. 1998) blocked adherence of P-fimbriated E. coli to uroepithelial cells. The structures of these compounds were determined by nuclear magnetic resonance (NMR) analysis to be polyflavan-3-ol or PAC trimers composed of epicatechin units with an A-type linkage (Foo et al. 2000). The A-type linkage between monomer units (Figure 6.3), which features two linkage sites between the units (4β→8 and 2β→O→7 interflavanoid bonds), is a structural feature common to PACs from Vaccinium fruit. Proanthocyanidins from most other sources including cocoa and grape seeds contain primarily B-type (4β→8) linkages (Neto 2007), as shown in Figure 6.3. Cranberry PACs are primarily dimers, trimers, and larger oligomers of epicatechin units containing both A- and B-type linkages; therefore, their three-dimensional structures are diverse. Although cranberry NDM has been cited more in recent studies for its ability to inhibit cellular adhesion of H. pylori (Burger et al. 2000) and the development of lymphoma (Hochman et al. 2008), no information is available on the molecular structures of its components.

The A-type linkage may hold the key to the antiadhesion activity of cranberry PACs. A comparison study of PACs isolated from several food sources, including cranberry, apple, grape, green tea, and chocolate, found that cranberry PACs prevented E. coli adhesion at the lowest concentration tested (60 μg/mL). Among the others, grape PACs showed antiadherence properties, but only at a much higher dose (1200 μg/mL), and the other food sources showed no activity (Howell et al. 2005). This study further found antiadhesion activity in human urine following consumption of cranberry juice cocktail, but not after consumption of other PAC sources. A randomized, double-blind, placebo-controlled crossover trial with 20 healthy volunteers was conducted to determine whether urine collected after cranberry consumption inhibited UPEC adherence to uroepithelial bladder cells. Subjects received a single dose of cranberry juice (750 or 250 mL mixed with 500 mL water) or placebo at night, and urine was collected in the morning and screened for antiadhesion against six UPEC strains. A significant and dose-dependent decrease in adherence of bacteria was observed in the urine of subjects who consumed cranberry (Di Martino et al. 2006).

Several studies have examined the phenomenon of antibacterial adhesion by cranberry constituents in an attempt to better understand what happens at a submicroscopic level. Atomic force microscopy was used to measure the effect of cranberry juice exposure on bacterial surface characteristics and adhesion forces in a P-fimbriae-expressing E. coli (HB101pDC1) and a nonfimbriated strain. A decrease in fimbrial length was measured after a short exposure to cranberry juice, with a greater biopolymer density recorded near the cell wall (Liu et al. 2006). Adhesion forces decreased in proportion to cranberry juice concentration. No significant effect of cranberry on surface polymers or adhesive forces was observed with nonfimbriated E. coli. Later studies by this group showed that the effects of culturing the bacteria in cranberry juice or PAC extract on bacterial adhesion forces were reversible (Pinzon-Arango, Liu, and Camesano 2009). Interestingly, a more marked decrease in adhesive forces was observed with the juice cocktail than with the PAC fraction, suggesting that other components in the juice play a role in reducing bacterial adhesion. Another study found that morphological changes occurred when E. coli bacteria were grown in the presence of cranberry juice or PAC extract (Johnson et al. 2008). The authors observed a decrease in visible P-fimbriae and downregulation of gene expression associated with flagellar basal body rod and motor proteins.

Clinical studies have demonstrated the efficacy of consuming cranberry juice or solids in UTI prevention for various populations, including women with recurrent UTIs (Walker et al. 1997; Stothers 2002; Bailey et al. 2007), pregnant women, (Wing et al. 2008), the elderly (Avorn et al. 1994; McMurdo et al. 2005; McMurdo et al. 2009), and children (Ferrara et al. 2009). A randomized, double-blind, placebo-controlled study of women aged 28–44 years with recurring UTIs using 400 mg of cranberry solids or placebo for 3 months found that 70% of the subjects had fewer UTIs while on cranberry (Walker et al. 1997). In a study of women aged 25–70 years with a history of high UTI recurrence (six or more in the previous year), it was found that consumption of 200 mg of concentrated cranberry extract standardized to 30% phenolics twice a day for 12 weeks prevented UTI recurrence in all subjects for the duration of the study (Bailey et al. 2007). A follow-up study of these subjects 2 years later found that those who continued to take cranberry remained free of infection. In pregnant women, for which asymptomatic bacteriuria can cause adverse perinatal outcomes if not detected and treated, consumption of 27% cranberry juice cocktail (240 mL, thrice daily) resulted in 57% reduction in asymptomatic bacteriuria and 41% reduction in UTI (Wing et al. 2008).

In addition to Avorn's landmark study on elderly women (Avorn et al. 1994), a randomized, double-blind, placebo-controlled study of hospitalized patients over 60 years of age found that the group consuming 25% cranberry juice cocktail (150 mL twice daily for 35 days or until discharge) had approximately half the occurrence of symptomatic UTI (McMurdo et al. 2005). A follow-up study by this group comparing treatment with 500 mg of cranberry extract to that with low doses of the antibiotic trimethoprim in older women found that both treatments reduced recurrent UTI. Trimethoprim treatment had only a limited advantage in this regard, and the cranberry group reported less adverse effects (McMurdo et al. 2009). Further clinical studies in such populations continue to provide information for health practitioners on the most effective forms and dosing regimens of cranberry against UTI.

6.9 FUTURE DIRECTIONS FOR RESEARCH ON CRANBERRIES AND DISEASE

Evidence from in vitro, in vivo, and clinical studies suggests that cranberry and its phytochemicals may have a mitigating effect on UTIs, cardiovascular diseases, and cancer. Mechanisms of action of cranberry range from antioxidative and anti-inflammatory actions to induction of cellular apoptosis; modulation of protein synthesis and gene expression involved in cell proliferation; prevention of bacterial adhesion and formation of biofilms that lead to infection; and effects on plasma lipoprotein levels, antioxidant status, and glucose metabolism. Future research should continue to examine cranberry's role in regulating these processes and address how the unique blend of phytochemicals found in cranberry fruit and juice works together. Cranberry's efficacy depends largely on the bioavailability of its phytochemicals to various tissues, which is another topic for further research.

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