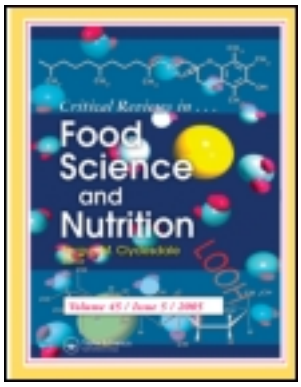

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Phytochemicals of Cranberries and Cranberry Products: Characterization, Potential Health Effects, and Processing Stability

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Phytochemicals of Cranberries and Cranberry Products: Characterization, Potential Health Effects, and Processing Stability

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Emerging evidence is elucidating how non-nutrient phytochemicals underlie the health promotion afforded by fruits and vegetables. This review focuses on Vaccinium macrocarpon*, the American cranberry, compiling a comprehensive list of its known phytochemical components, and detailing their prevalence in cranberry fruit and its products. Flavonoids, especially colored anthocyanins, abundant flavonols, and unique proanthocyanidins, have attracted major research attention. Other notable active components include phenolic acids, benzoates, hydroxycinnamic acids, terpenes and organic acids. Health effects of cranberries, cranberry products, and isolated cranberry components in humans and animals, as well as in vitro, are debated. Evidence for protection from several bacterial pathogens, cancer, cardiovascular disease, and inflammation is compelling, while neuroprotection and anti-viral activity also have begun to draw new consideration. Emerging bioavailability data is considered and potential molecular mechanisms are evaluated, linking phytochemicals to health effects through their biochemical properties and reactions. Finally, the effects of processing and storage on cranberry phytochemicals is discussed, with a focus on identifying research gaps and novel means to preserve their natural, health-promoting components.*

Keywords anthocyanins, proanthocyanidins, flavonols, phenols, urinary tract infections, cancer, inflammation, absorption

INTRODUCTION

Everyone grows up hearing the admonitions of mothers to "eat your fruits and vegetables," and ample scientific evidence now exists that our mothers were justified. Indeed, the health benefits from fruits and vegetables are among the best demonstrated in nutrition. Increased dietary consumption of fruits and vegetables correlates with increased cardiovascular health as well as reduced cancer, stroke, degenerative diseases, loss of functionality associated with aging, and more (Ames and Gold, 1991; Block et al., 1992; Ames et al., 1993; Joshipura et al., 1999; Temple, 2000; Feldman, 2001; Liu, 2003; Dai et al., 2006). While fruits and vegetables are rich sources of vitamins and minerals, recent attention has focused heavily on the effects of phytochemical components such as flavonoids, stilbenes, nonnutritive carotenoids, phytoestrogens, terpenes and other diverse phenolics (Schaich and Fisher, 1993; Liu, 2004; Rice-Evans, 2004; Ahuja et al., 2006; Baur and Sinclair, 2006; Cirico and Omaye, 2006; He and Liu, 2006). The exact mechanisms of phytochemical action remain largely unexplained and are currently the subject of much speculation and research. Antioxidant mechanisms have been proposed, especially in the context of cardiovascular health, cancer and age-related degenerative diseases (Hollman, 2001; Liu, 2003; Liu, 2004; Shukitt-Hale et al., 2006a; Patel et al., 2007; Srinivasan et al., 2007). For inflammation and cancer in particular, phytochemical interactions with vital proteins, signal transduction pathways, and pathogen binding also have attracted significant attention (Puupponen-Pimiä et al., 2001; Steinberg et al., 2004; Comalada et al., 2005; Baur and Sinclair, 2006; Liu et al., 2006; Neto, 2007a; Ruel and Couillard, 2007).

Looking at one fruit in particular, the American cranberry, species *Vaccinium macrocarpon*, has for centuries been considered a health food. Native Americans relied on cranberries as a food, a meat preservative, and a medicine to treat diverse

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ailments, and they introduced them to European settlers who enjoyed the bright red berries at the first Thanksgiving dinner (Henig and Leahy, 2000). No longer merely a side dish to be eaten with turkey at Thanksgiving, cranberries today are an increasingly significant crop in the United States. With an almost 50% increase in per capita consumption between 1989 and 2004 (USDA-ERS, 2007), the U.S. cranberry crop topped 660 million pounds worth ~$1.5 billion in 2006. This increased demand for and the corresponding production of the fruit have been stimulated by widespread new emphasis on consuming foods that promote health and well-being and by the perceived unique health benefits associated with cranberries.

Cranberry juice was a staple folk medicine prescription for healing urinary tract infections in women, and recent verification of this pharmaceutical effectiveness against bacterial pathogens in contemporary clinical studies (see Section titled Urinary Tract Infections) has excited an explosion of research into cranberry chemical composition, health effects, absorption, bioavailability, metabolism, and antioxidant capacity. However, despite targeted grant programs, dozens of studies, and several theories, no mechanism to explain the effects of cranberries on urinary tract infections has yet been conclusively identified. Indeed, as new data becomes available, often more questions than answers arise.

To provide a basis for understanding the complexities of cranberry nutraceutical actions, this paper compiles current information about the phytochemical composition of American cranberries and reviews cranberry health effects in the context of the absorption, bioavailability, metabolism, and excretion of cranberry phytochemicals. Molecular mechanisms proposed to account for the potential dietary benefits of cranberries are discussed. Lastly, because cranberries are rarely consumed in their natural state, processing critically influences components available to affect the health of consumers; thus, alterations in composition and destruction of nutrients and phytochemicals by processing and storage also are reviewed. Consideration is limited to the American cranberry; its wild relative (the European cranberry), *Vaccinium Oxycoccus*, will not be included.

PHYTOCHEMICALS OF CRANBERRIES AND CRANBERRY PRODUCTS

Cranberries are a uniquely rich and heterogeneous source of phytochemicals. Currently, over 150 individual phytochemicals have been identified and studied in cranberries, although undoubtedly many more will be discovered with continued improvement in analytical methods.

Table 1 summarizes general classes of phytochemicals reported to exhibit bioactivity in cranberries, along with their characteristic properties, functionalities, and reported health effects. By far, the dominant components are flavonoids. Most readily-recognized are anthocyanins responsible for the bright red cranberry color; flavonols, secondary yellowish pigments; proanthocyanidins associated with protection against urinary tract infections; catechins, organic acids, and resveratrol which contribute the sour, astringent flavor unique to cranberries; terpene aroma components; and pectins that gel cooked cranberries into the cranberry sauce familiar to everyone.

Table 2 provides a comprehensive list of individual cranberry phytochemicals in each class, along with their concentrations in cranberries and cranberry products. Most research has focused on cranberry flavonoids, including anthocyanins (ACYs), proanthocyanidins (PACs), flavonols, and flavan-3-ols. Other polyphenolics, simple phenolics, terpenes, organic acids, complex carbohydrates, and sugars of cranberries have attracted somewhat less medical research attention, but nevertheless have shown some promise as health promoters.

Additional details about cranberry components and their health effects are provided in the following discussion.

Anthocyanins

Anthocyanins (ACYs), probably the most studied chemical component of cranberries, are responsible for the majority of the red color that is appealing and distinctive enough to demand its own shade: "cranberry red". Localized within color bodies in the exocarp layer of the fruit's skin, anthocyanins are potent color attractants in cranberries and accumulate as the fruit matures (Sapers et al., 1983a; Vvedenskaya and Vorsa, 2004). Given the strong antioxidant activity of anthocyanins, recent research focus has shifted from spectral characteristics to bioactivity and potential health benefits. Structures of individual anthocyanins are shown in Table 3; corresponding concentrations in whole berries and in cranberry juice concentrate are listed in Table 2.

The major pigments of cranberries, 3-monogalactosides and 3-monoarabinosides of cyanidin and peonidin (Structures **1–2** and **4–5**, respectively, Table 3), were identified first (Sakamura and Francis, 1961; Zapsalis and Francis, 1965) and the 3-monoglucosides of cyanidin (structure **3**, Table 3) and peonidin (structure **6**, Table 3) were identified a short time later (Fuleki and Francis, 1967). For several decades, these six anthocyanins remained the only ones found in cranberries and are still believed to contribute most of the desirable color in fresh cranberries.

Recent analyses have revealed a much more diverse ACY profile in processed cranberry products. Using HPLC-ESI-MS-MS, Wu and Prior (2005) identified 13 anthocyanins in freeze dried cranberries, adding malvidin, pelargonidin, delphinidin, and petunidin to the basic backbone structures (along with cyanidin and peonidin). Furthermore, their results showed that of the eighteen anthocyanin-rich fruits tested, only cranberries and Concord grapes contained all six anthocyanin backbones. Of the thirteen anthocyanins identified, all except peonidin 3,5-diglucoside are present also in processed cranberry juice (Ohnishi et al., 2006). However, the possibility that these newly identified anthocyanins are artifacts of tandem mass spectral analysis cannot yet be ruled out and confirmatory identification

Table 1 Summary of phytochemical classes with potential health benefits from cranberry fruit

Phytochemical Class	Example	Function in Plant	Function in Cranberry Products	Potential Health Benefits	Bio-availability	References
Flavonoids:						
Anthocyanins	peonidin-3-*O*-galactoside	color attractants	primary red pigment	AC, AOX, NFP, CHB	+	Karakaya, 2004; Andres-Lacueva et al., 2005; Crews et al., 2005 Yao and Viero, 2006; Ohnishi et al., 2006
Flavonols	quercetin-3-*O*−galactoside	color attractants, reproduction, UV protection	copigment, AOX stabilizer	AC, AI, AOX, CHB	++	Moon et al., 2001; Yan et al., 2002; Zhang and Wang 2003; Li et al., 2004; Vvendenskaya and Vorsa, 2004; Karakaya, 2004; Chen and Zuo, 2007
Flavanols (Catechins)	epicatechin	pathogen defense	astringent flavors, AOX stabilizer	AC, AI, AOX, CHB	++	Manach et al., 1999; Chen et al., 2001; Cunningham et al., 2004; Karakaya, 2004; Harnley et al., 2006
Proanthocyanidins	proanthocyanin A2	pathogen defense, structural	astringent flavors, AOX stabilizer	AA, AOX, AU, AV, CHB, NFP	−	Howell et al., 1998; Foo et al., 2000a; Foo et al., 2000b; Ariga, 2004; Prior and Gu, 2005; Neto et al., 2006
Polymeric Color Compounds	cyanidin-pentoside-flavan-3-ol	color attractants	red-brown pigment	AOX?, AA?, AV?	?	Hong and Wrolstad, 1986a; Reed et al., 2005
Non-Flavonoids:						
Non-Flavonoid Polyphenols	resveratrol	diverse	astringent flavors, AOX stabilizer	AC, AI, AOX	+	Daniel et al.,1989; Mazur et al., 2000; Asensi et al., 2002; Wang et al., 2002; Baur and Sinclair, 2006
Simple Phenolics	salicylic acid	AOX, odor attractants	odors, AOX stabilizer	AC, AI, AOX, CHB	++	Borne and Rice-Evans, 1998; Zuo et al., 2002; Zhang and Zuo, 2004; Karakaya, 2004; Duthie et al., 2005
Non-Phenols:						
Non-Aromatic Organic Acids	ascorbic acid	antibacterial	sour flavors	AC, AOX	+++	Hong and Wrolstad, 1986a; Jensen et al., 2002; Cunningham et al., 2004; He and Liu, 2006
Complex Carbohydrates	pectin	structural	gelation, edible films	AC, AOX	−	Struckrath et al., 1998; Cunningham et al., 2004; Kahlon and Smith, 2006
Sugars	fructose	energy storage	sweet flavors	AA, AC	+++	Hong and Wrolstad 1986a; Neto et al., 2005; He and Liu, 2006

Abbreviations Used: AA = Antiadhesion towards bacteria, AC = Anticancer, AI = Anti-inflammatory, AOX = Antioxidant, AU = Antiulcer, AV = Antiadhesion towards viral pathogens, CHB = Cardiovasular health benefits, NFP = Neural Function Protection. Symbols used: ? denotes unknown.

by NMR, IR, or other non-destructive analytical or chemical techniques is needed. Furthermore, these have not been identified in fresh fruit and could be a result of processing or sample preparation. The malvidin, pelargonidin, delphinidin and petunidin compounds account for only about 1% of the total ACYs of cranberries (Wu et al., 2006) and thus do not contribute significantly to cranberry color. If these novel cranberry anthocyanins do result from processing, questions arise as to whether they add to or detract from the nutraceutical properties of fresh cranberries. Natural or not, it will be interesting to learn whether they have some specific and unique health benefits (Wu et al., 2006).

Although cranberries are rich in dietary anthocyanins in general, they may be even more important as major sources of individual ACY species in the US diet. Peonidin glycosides, in particular, make up only ∼7% of the total ACY intake in the US (Wu et al., 2006) but comprise half of the total cranberry anthocyanins (Prior et al., 2001). Also notable are the cyanidin and peonidin galactosides and arabinosides, which account for 57.8% and 39.5%, respectively, of cranberry anthocyanins (Prior et al., 2001) but are rarely found in other commonly consumed fruits (Wu and Prior, 2005). New evidence suggests that these glycosides appear to selectively facilitate absorption of anthocyanins (Mulleder et al., 2002; Vorsa et al., 2007a), so

Table 2 Phytochemical components of the American cranberry, Vaccinium macrocarpon

Phytochemical/Class	#	Conc. in Cran.	Conc. in Cran. Products*	References
Anthocyanins		13.6–171 mg/100 g	13.6 mg/L	Sapers et al., 1983; Ozgen et al., 2005 Lee et al., 2005;
cyanidin-3-*O*-galactoside	1	—	2.0 mg/L	Cunningham et al., 2004
cyanidin-3-*O*-arabinoside	2	—	1.4 mg/L	Cunningham et al., 2004
cyanidin-3-*O*-glucoside	3	—	0.1 mg/L	Cunningham et al., 2004
peonidin-3-*O*-galactoside	4	—	2.8 mg/L	Cunningham et al., 2004
peonidin-3-*O*-arabinoside	5	—	1.1 mg/L	Cunningham et al., 2004
peonidin-3-*O*-glucoside	6	—	0.3 mg/L	Cunningham et al., 2004
peonidin 3,5-digalactoside		—	—	Wu and Prior, 2005
malvidin-3-*O*-arabinoside	7	—	—	Wu and Prior, 2005; Ohnishi et al., 2006
malvidin-3-*O*-galactoside	8	—	—	Wu and Prior, 2005; Ohnishi et al., 2006
pelargonidin-3-*O*-arabinoside	9	—	—	Wu and Prior, 2005; Ohnishi et al., 2006
pelargonidin-3-*O*-galactoside	10	—	—	Wu and Prior, 2005; Ohnishi et al., 2006
delphinidin-3-*O*-arabinoside	11	—	—	Wu and Prior, 2005; Ohnishi et al., 2006
petunidin-3-*O*-galactoside	12	—	—	Wu and Prior, 2005; Ohnishi et al., 2006
Polymeric Color Compounds		—	—	Hong and Wrolstad, 1986a; Reed et al., 2005
Flavonols		200–400 mg/kg	48.5 mg/L	Zheng and Wang, 2003; Cunningham et al., 2004; Vvedenskaya and Vorsa, 2004
quercetin	13	104 mg/kg**; 194 mg/kg**; 41.6 μg/g	13.0 mg/L; 30.6 μmol/L	Zheng and Wang, 2003;Cunningham et al., 2004; Harnley et al., 2006; Mullen et al., 2007
quercetin-3-galactoside (hyperin)	14	70.4 μg/g	23.2 mg/L	Zheng and Wang, 2003; Cunningham et al., 2004
quercetin-3-α-arabinopyranoside (avicularin)	15	34.4 μg/g	1.8 mg/L; 5.4 μmol/L	Zheng and Wang, 2003; Cunningham et al., 2004; Vvedenskaya et al., 2004; Mulllen et al., 2007
quercetin-3-rhamnoside (quercitrin)	16	41.6 μg/g	5.2 mg/L; 10.8 μmol/L	Zheng and Wang, 2003; Cunningham et al., 2004; Vvedenskaya et al., 2004; Mullen et al., 2007
quercetin-3-xyloside		—	6.0 μmol/L	Yan et al., 2002; Mullen et al., 2007
quercetin-3-β-glucoside (isoquercetin)		—	—	Vvedenskaya et al., 2004; Vvedenskaya and Vorsa, 2004
3-methoxyquercetin-3-β-galactoside		—	—	Vvedenskaya et al., 2004; Vvedenskaya and Vorsa, 2004
3′-methoxyquercetin-3-α-xylopyranoside			—	Vvedenskaya et al., 2004; Vvedenskaya and Vorsa, 2004
quercetin-3-*O*-(6″-p-coumaroyl)-β-galactoside	17	—	—	Vvedenskaya et al., 2004; Vvedenskaya and Vorsa, 2004
quercetin-3-*O*-(6″-benzoyl)-β-galactoside	18	—	—	Vvedenskaya et al., 2004; Vvedenskaya and Vorsa, 2004
kaempferol		—	—	Bilyk and Sapers, 1986
kaempferol-3-glucoside		5.6 μg/g	—	Zheng and Wang, 2003
myricetin	19	69 mg/kg**; 166 mg/kg**	5.3 mg/L; 16.1 μmol/L	Cunningham et al., 2004; Harnley et al., 2006; Mullen et al., 2007
myricetin-3-β-xylopyranoside	20	—	3.3 μmol/L	Vvedenskaya et al., 2004; Vvedenskaya and Vorsa, 2004, Mullen et al., 2007
myricetin 3-α-arabinofuranoside	21	37.5 μg/g	—	Yan et al., 2002; Zheng and Wang, 2003
Catechins		7.3 mg/100 g	6 mg/L	Gu et al., 2004
(−)epicatechin	22	4.5 mg/100 g**	8.1 mg/L (WJ); 3.5 mg/L	Gu et al., 2004a; Cunningham et al., 2004; Harnley et al., 2006
epigallocatechin		1.5 mg/100 g**	—	Harnley et al., 2006
(+)catechin	23	0.8 mg/100 g**	8.1 mg/L (WJ)	Chen et al., 2001; Harnley et al., 2006
epigallocatechin gallate		1.9 mg/100 g**	—	Harnley et al., 2006
catechin gallate		7.9 mg/100 g**	—	Harnley et al., 2006
gallocatechin gallate		0.4 mg/100 g**	—	Harnley et al., 2006
Proanthocyanins		418.8 mg/100 g	231–625 mg/L; 35 mg/100 g (CS); 80 mg/100 g (SDC)	Porter et al., 2001; Gu et al., 2004; Cunningamn et al., 2004, Neto et al., 2006
Total Dimers		25.9 mg/100 g	29 mg/L	Gu et al., 2004
procyanidin B2 {(EC-(4$\beta\rightarrow$8)-EC}	24	—	—	Foo et al., 2000a; Prior et al., 2001
procyanidin A2 {EC-(4$\beta\rightarrow$8, 2$\beta\rightarrow O\rightarrow$7)-EC}	25	—	—	Foo et al., 2000a; Prior et al., 2001
Total Trimers		18.8 mg/100 g	17 mg/L	Gu et al., 2004
EC-(4$\beta\rightarrow$6)-EC-(4$\beta\rightarrow$8, 2$\beta\rightarrow O\rightarrow$7)-EC	26	—	—	Foo et al., 2000a; Prior et al., 2001

Table 2 Phytochemical components of the American cranberry, Vaccinium macrocarpon (*Continued*)

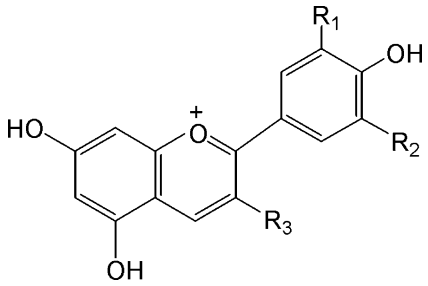
Phytochemical/Class	#	Conc. in Cran.	Conc. in Cran. Products*	References
EC-(4β→8, 2β→O→7)EC-(4β→8)-EC	27	—	—	Foo et al., 2000a; Prior et al., 2001
EC-(4β→8)-EC-(4β→8, 2β→O→7)-EC	28	—	—	Foo et al., 2000a; Prior et al., 2001
Total 4–6 mers		70.3 mg/100 g	49 mg/L	Gu et al., 2004
Total 7–10 mers		62.9 mg/100 g	41 mg/L	Gu et al., 2004
Total >10 mers		233.5 mg/100 g	89 mg/L	Gu et al., 2004
flavanones		—	—	—
naringenin 7-glucosode (Prunin)		—	—	Turner et al., 2005
Non-flavonoid Polyphenols		—	—	—
phloretin 2-glucoside (phloridzin)		—	—	Lotito and Frei, 2004; Turner et al., 2005
ellagic acid		120 mg/kg (DWB)	—	Daniel et al., 1989
2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate	29	—	—	Turner et al., 2007
trans-resveratrol	30	—	~ 0.2 mg/L (WJ)**	Wang et al., 2002; Baur and Sinclair, 2006
cis-resveratrol		—	~ 0.03 mg/L (WJ)**	Wang et al., 2002; Baur and Sinclair, 2006
secoisolariciresinol (SECO)	31	10.54 mg/kg (DWB)**	—	Mazur et al., 2000; Cunningham et al., 2004
Phenolic Acids and Benzoates				
Total phenolic Acids		5.7 g/kg	—	Zuo et al., 2002
benzoic acid	32	4741 μg/g**	54.94 ug/mL**; 43.7 mg/L	Zuo et al., 2002; Cunningham et al., 2004; Zhang and Zuo, 2004
o-hydroxybenzoic acid (salicylic acid)	33	23.2 μg/g**	3.11 μg/mL**; 7.04 mg/L**	Zuo et al., 2002; Zhang and Zuo, 2004; Duthie et al., 2005
m-hydroxybenzoic acid		9.14 μg/g**	0.15 μg/mL**	Zuo et al., 2002; Zhang and Zuo, 2004
p-hydroxybenzoic acid		21.6 μg/g**	0.07 μg/mL**	Zuo et al., 2002; Zhang and Zuo, 2004
p−hydroxyphenylacetic acid		7.36 μg/g**	ND	Zuo et al., 2002; Zhang and Zuo, 2004
2,3-dihydroxybenzoic acid		3.16 μg/g**	2.41 μg/mL**	Zuo et al., 2002; Zhang and Zuo, 2004
2,4-dihydroxy benzoic acid	34	42.5 μg/g**	ND	Zuo et al., 2002; Zhang and Zuo, 2004
3,4-dihydroxybenzoic acid (protocatechuic acid)		—	2.3 mg/L	Cunningham et al., 2004
vanillic acid	35	19.2 μg/g**; 49.3 μg/g	1.2 mg/L	Zuo et al., 2002; Zheng and Wang 2003; Cunningham et al., 2004
trans-cinnamic acid	36	20.5 μg/g**	0.18 μg/mL**	Zuo et al., 2002; Zhang and Zuo, 2004
o-hydroxycinnamic acid		89 μg/g**	3.43 μg/mL**	Zuo et al., 2002; Zhang and Zuo, 2004
p-coumaric acid	37	253.8 μg/g**	2.63 μg/mL**; 4.4 mg/L; 5.2 mg/L	Chen et al., 2001; Zuo et al., 2002; Zhang and Zuo, 2004
o-phthalic acid		15.7 μg/g**	—	Zuo et al., 2002
caffeic acid	38	156.4 μg/g**; 42.5 μg/g	1.1 mg/L	Zuo et al., 2002; Zheng and Wang 2003; Cunningham et al., 2004
ferulic acid	39	87.9 μg/g**	1.11 μg/mL**	Zuo et al., 2002; Zhang and Zuo, 2004
sinapic acid	40	211.8 μg/g**	5.11 μg/mL**	Zuo et al., 2002; Zhang and Zuo, 2004
gallic acid		**	—	Zhang and Shetty, 2000
3-O-caffeoylquinic acid (chlorogenic acid)		—	5.1 mg/L; 11.0 mg/L	Chen et al., 2001; Cunningham et al., 2004
5-O-caffeoylquinic acid			25.4 umol/L	Mullen et al., 2007
benzoic acid α-L-arabinopyranosyl (1→6)-β-D-glucopyranoside		—	—	He and Liu, 2006
6-O-benzoyl-β-D-glucose (vacciniin)		0.022%	—	Marwan and Nagel, 1986; Heimhuber et al., 1990; He and Liu, 2006
1-O-benzoyl-β-D-glucose		?	—	Heimhuber et al., 1990
2-O-benzoyl-β-D-glucose		?	—	Heimhuber et al., 1990
6-O-benzoyl-α-D-glucose		?	—	Heimhuber et al., 1990
Other Phenols		—	—	—
1-[3-(4-hydroxyphenyl)-2-propenoate]-β-D-glucopyranoside		—	—	He and Liu, 2006
benzyl benzoate		—	—	Croteau and Fagerson, 1971
benzaldehyde		—	—	Croteau and Fagerson, 1971
4-methoxy benzaldehyde		—	—	Croteau and Fagerson, 1971
benzyl alcohol		—	—	Croteau and Fagerson, 1971
2-phenyl ethanol		—	—	Croteau and Fagerson, 1971
dibutyl phthalate		—	—	Croteau and Fagerson, 1971
2-hydroxy diphenyl		—	—	Croteau and Fagerson, 1971
methyl benzoate		—	—	Croteau and Fagerson, 1971

(*Continued on next page*)

Table 2 Phytochemical components of the American cranberry, Vaccinium macrocarpon (*Continued*)

Phytochemical/Class	#	Conc. in Cran.	Conc. in Cran. Products*	References
ethyl benzoate		—	—	Croteau and Fagerson, 1971
benzyl formate		—	—	Croteau and Fagerson, 1971
benzyl ethyl ether		—	—	Croteau and Fagerson, 1971
acetophenone		—	—	Croteau and Fagerson, 1971
Non-Phenolic Organic Acids		2.67–3.65% (w/v)	~ 2.75%(WJ)	Jensen et al., 2002; He and Liu, 2006
quinic acid	41	—	1.05% (WJ), >0.26%	Cunningham et al., 2004; He and Liu, 2006
citric acid		—	1.06% (WJ)	Jensen et al., 2002; Cunningham et al., 2004
malic acid		—	0.78% (WJ)	Cunningham et al., 2004
shkimic acid	42	—	0.1–0.9 g/100 g (WJ)	Hong and Wrolstad, 1986a; Jensen et al., 2002
galacturonic acid		—	0.19% (WJ)	Cunningham et al., 2004
2-furoic acid		—	2.9 ppm	Cunningham et al., 2004
oxalic acid		—	5 ppm	Cunningham et al., 2004
2(R)-hydroxybutanedioic acid 1-methyl ester (tartaric acid methyl ester)	43	—	—	He and Liu, 2006
2(R)-hydroxybutanedioic acid (tartaric acid)		—	—	He and Liu, 2006
fumaric acid		—	—	Seeram et al., 2004
isocitric acid		—	—	Seeram et al., 2004
2-methyl butyric acid		—	—	Duke, 1992
ascorbic acid		11.5 mg/100 g	0–2 mg/100 g (WJ)	Licciardello et al., 1952; Hong and Wrolstad, 1986a; Cunningam et al., 2004
Terpenes		—	—	Jensen et al., 2002; Choi et al., 2005
oleanolic acid		—	—	Wu and Parks, 1953
ursolic acid	44	60–110 mg/100 g	—	Wu and Parks, 1956; Kondo, 2006; He and Liu, 2006
cis-3-*O*-*p*-hydroxy cinnamoyl ursolic acid	45	—	—	Murphy et al., 2003; He and Liu, 2006
trans-3-O-*p*-hydroxy cinnamoyl ursolic acid	46	—	—	Murphy et al., 2003; He and Liu, 2006
β-sitosterol		—	—	He and Liu, 2006
β-sitosterol-3-$O-\beta$-D-glucoside		—	—	He and Liu, 2006
monotropein	47	—	—	Jensen et al., 2002; Choi et al., 2005
6,7-dihydromonotropein	48	—	—	Jensen et al., 2002
10-*p*-*cis*-coumaroyl-1*S*-dihydromonotropein	49	—	—	Turner et al., 2007
10-*p*-*trans*-coumaroyl-1*S*-dihydromonotropein		—	—	Turner et al., 2007
Complex Carbohydrates/Fiber		12000–160000 ppm	0.1% (WJ); 1.4% (CS)	Marlett and Vollendorf, 1994; Cunningham et al., 2004; Kahlon and Smith, 2007
cellulose		—	—	Holmes and Rha, 1978
hemicellulose		—	—	Holmes and Rha, 1978
protopectin		—	—	Stuckrath et al., 1998
high methoxy pectin		—	—	Stuckrath et al., 1998
Sugars		—	3.7–5.4% (WJ)	Hong and Wrolstad, 1986a; Lowe and Fagelman, 2001; Cunningham et al., 2004, Turner et al., 2005
glucose		—	4.3%	Hong and Wrolstad, 1986a; Cunningham et al., 2004
sucrose		—	<0.05%	Cunningham et al., 2004
fructose		—	0.8% (WJ)	Hong and Wrolstad, 1986a; Lowe and Fagelman, 2001; Cunningham et al., 2004
Sorbitol		—	trace	Cunningham et al., 2004
1-*O*-methylgalactose		—	5 ppm (WJ)	Turner et al., 2005
Miscellaneous				
lutein		0.28–2 ppm	—	Duke, 1992
niacin		1–8.3 ppm	—	Duke, 1992
pantothenic acid		2.2–16 ppm	—	Duke, 1992
thiamin (vitamin B1)		0.3–2.5 ppm	—	Duke, 1992
riboflavin (vitamin B2)		0.2–1.7 ppm	—	Duke, 1992
adermine (vitamin B6)		0.6–5.4 ppm	—	Duke, 1992
folic acid (vitamin B9)		0.1–0.2 ppm	—	Duke, 1992
beta-carotene		0.2–2.6 ppm	—	Duke, 1992
alpha tocopherol		9–81 ppm	—	Duke, 1992

Table 3 Chemical structures of anthocyanins found in cranberries



Structure #	Anthocyanin Name	R_1	R_2	R_3
1	cyanidin-3-*O*-galactoside	OH	H	Galactose
2	cyanidin-3-*O*-arib inoside	OH	H	Arabinose
3	cyanidin-3-*O*-glucoside	OH	H	Glucose
4	peonidin-3-*O*-galactoside	OMe	H	Galactose
5	peonidin-3-*O*-arib inoside	OMe	H	Arabinose
6	peonidin-3-*O*-glucoside	OMe	H	Glucose
7	malvidin-3-*O*-arib inoside	OMe	OMe	Arabinose
8	malvidin-3-*O*-galactoside	OMe	OMe	Galactose
9	pelargonidin-3-*O*-arib inoside	H	H	Arabinose
10	pelargonidin-3-*O*-galactoside	H	H	Galactose
11	delphinidin-3-*O*-arib inoside	OH	OH	Arabinose
12	petunidin-3-*O*-galactoside	OMe	OH	Galactose

may increase the availability of cranberry anthocyanins relative to other sources.

Whole raw cranberries contain high but inconsistent levels of anthocyanins, varying from 13.6 to 140 mg/100 g depending on fruit size, ripeness, variety, and other factors (Sapers et al., 1983a, 1983b; Vorsa and Welker, 1985; Özgen et al., 2005). These high levels make cranberries a potentially significant dietary source of anthocyanins, especially considering that current U.S. per capita consumption of anthocyanins is estimated at only 12.5 mg total ACY/day (Wu et al., 2006). Interestingly, it is common industry practice for cranberry processors to pay growers a "color bonus" based on ACY content of their berries (Francis, 1995; Vvedenskaya and Vorsa, 2004; Özgen et al., 2005), and much effort has been devoted to improving the anthocyanin yield (and hence color intensity) of cranberries, both through plant breeding techniques and use of topical treatments (Vorsa et al., 2003; Özgen et al., 2005). Indeed, very recently, a new cultivar of cranberry has been bred and patented, claiming higher anthocyanin yields as one of its primary improvements over traditionally cultivated strains (Vorsa, 2007).

Cranberry juice cocktails contain lower amounts of anthocyanins, ranging from approximately 1.2–1.5 mg/100 mL (Prior et al., 2001; Lee et al., 2005) to as high as 2.5 mg/100 mL (Cunningham et al., 2004).

Polymeric Pigments

While monomeric anthocyanins are the major bright red pigments, polymeric anthocyanin-containing compounds are the source of deeper brownish-red colors in cranberries. About 10% of the color in freshly prepared whole cranberry juice is due to polymeric material (Hong and Wrolstad, 1986a), and this increases with age of the juice. In fact, cranberry researchers measure polymeric color (determined as the color remaining after anthocyanins are bleached) to determine the extent of color deterioration during post-harvest degradation, processing, and storage.

Pigmented polymers from cranberries have been only partially characterized and remain poorly understood. Nevertheless, several lines of evidence support their existence. Some cranberry proanthocyanidin fractions show a weak absorbance at 520 nm, the wavelength associated with anthocyanins (Porter et al., 2001). Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) analysis of cranberry juice extract detected the presence of novel anthocyanin-epicatechin oligomers (Neto, 2007b), as well as several vinyl linked ACY-flavanol dimers (Krueger et al., 2004; Reed et al., 2005):

cyanidin-pentoside-flavan-3-ol (m/z 735.3)
peonidin-pentoside-flavan-3-ol (m/z 749.3)
cyanidin-hexoside-flavan-3-ol (m/z 765.4)
peonidin-hexoside-flavan-3-ol (m/z 779.3)

Several anthocyanins linked to A and B type PAC dimers were also identified (Krueger et al., 2004; Reed et al., 2005):

cyanidin-pentoside-DP2 (A-type = m/z 1021.2, B-type = m/z 1023.1)
peonidin-pentoside-DP2 (A-type = m/z 1035.1, B-type = m/z 1037.1)
cyanidin-hexoside-DP2 (A-type = m/z 1051.2, B-type = m/z 1053.2)
peonidin-hexoside-DP2 (A-type = m/z 1065.2, B-type = m/z 1067.1).

Twenty-eight similar ACY-PAC conjugates in spray dried juice and twenty-five in whole cranberry berry extracts were reported, all with a single anthocyanin residue and degrees of polymerization up to six (Krueger et al., 2004). Increased heterogeneity of higher polymers prevented their characterization (Krueger et al., 2004). One plausible chemical structure for these polymers is presented in Fig. 1. The authors propose that these compounds are generated during normal fruit ripening, although the possibility that the polymers were analytical artifacts or chemical degradation products could not be ruled out. MALDI-MS characterizations are tentative, and NMR or IR confirmation will be necessary for positive identification of anthocyanin and flavanol monomer units, sugar moieties, linkages involved, and overall structures.

Substantial research on polymeric color has been conducted in wine, where anthocyanins can be linked to flavan-3-ol monomers and polymers via a vinyl linkage (from acetaldehyde condensation) or via an ether linkage (through direct condensation) (Es-Safi and Cheynier, 2004; Sun and Hai Liu, 2006). In grapes, both types of polymers contain multiple units of

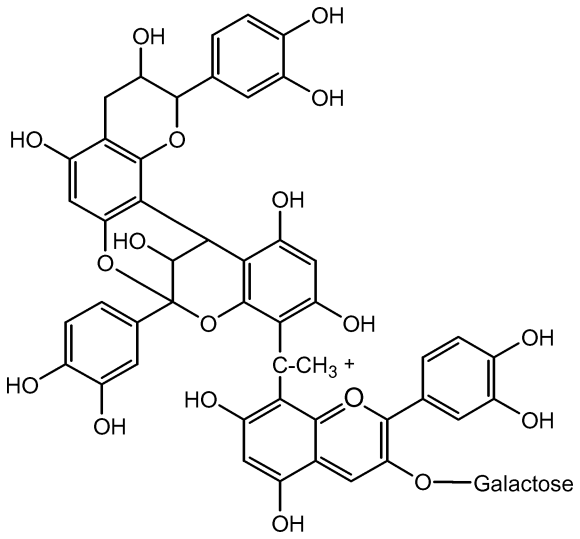


Figure 1 Postulated vinyl linkages between anthocyanin (cyanidin-3-galactoside) and proanthocyanidin (PAC A2) in a polymeric pigment from cranberries, determined by MALDI-TOF mass spectral analysis (Krueger et al., 2004; Reed et al., 2005).

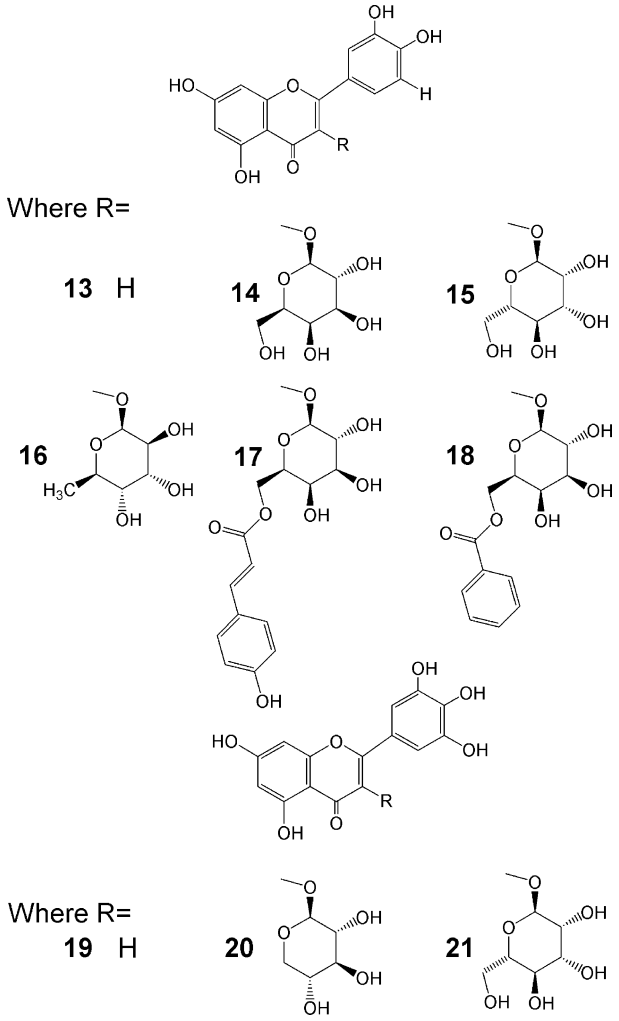


Figure 2 Chemical structures of selected cranberry flavonols (quercetin and derivatives **13**–**18**; myricetin and derivatives **19–21**): quercetin (**13**); hyperin (**14**); avicularin (**15**); quercitrin (**16**); quercetin-3-*O*-(6″-*p*-coumaroyl)-β-galactoside (**17**); quercetin-3-*O*-(6″-benzoyl)-β-galactoside (**18**); myricetin (**19**); myricetin β-xylopyranoside (**20**) and myricetin 3-α-arabinoside (**21**).

epicatechin and one unit of malvidin-3-glucoside. Wine also contains a class of anthocyanin-derived pigments known as pyranthocyanins (Sun and Hai Liu, 2006). However, only vinyl-linked anthocyanin-flavanol complexes have been found in cranberries.

Given their strong antioxidant activity and structural similarities to PACs, polymeric color compounds warrant research as health-promoting compounds. With high molecular weight, these polymeric color compounds (and perhaps ether-linked ACY-PAC complexes) are likely to be present in significant quantity in cranberry NDM (non-dialyzable material) (see Section titled High Molecular Weight Fraction (NMD)) and may be responsible for some health effects observed from that cranberry fraction. However, support for any health benefits necessitates further research into the fate of these molecules upon consumption, which is totally absent in today's literature. Health-promoting activity in the mouth, stomach, and the digestive tract should be expected, but the high molecular weight precludes significant absorption: systemic effects are feasible only if these compounds are degraded to smaller compounds in the GI tract.

Flavonols

Flavonols in whole cranberries are mostly glycosylated forms of quercetin, myricetin, and, to a lesser extent, kaempferol. Puski and Francis (1967) first identified individual flavonols and flavanol glycosides in cranberries and found quercetin (structure **13**, Fig. 2), quercetin-3-galactoside (hyperin or hyperoside) (structure **14**, Fig. 2), quercetin-3-arabinoside (avicularin) (structure **15**, Fig. 2), quercetin-3-rhamnoside (quercitrin) (structure **16**, Fig. 2), myricetin-3-arabinoside (structure **21**, Fig. 2) and myricetin-3-digalactoside in the Early Black variety of cranberries. Later, small amounts of kaempfero1 (0 to $\sim$2.5 mg/kg) were reported in some, but not all, cranberry varieties (Bilyk and Sapers, 1986).

Contemporary analytical technology has revealed a far more diverse profile of cranberry flavonols. Myricetin 3-α-arabinofuranoside, quercetin 3-xyloside, and 3-methoxyquercetin 3-β-galactoside (isorhamnetin-3-galactoside) have been identified by ^{1}H and ^{13}C NMR (Yan et al., 2002). Zheng and Wang (2003) reported the presence of kaempferol-3-glucoside and another unidentified kaempferol derivative in Ben Lear cranberries. HPLC isolated and MS, ^{1}H and ^{13}C NMR identified quercetin-3-β-glucoside (isoquercetin), 3′-methoxyquercetin-3-α-xylopyranoside, quercetin-3-O-(6″-p-coumaroyl)-β-galactoside (structure **17**, Fig. 2), quercetin-3-O-(6″-benzoyl)-β-galactoside (structure **18**, Fig. 2) and myricetin-3-β-xylopyranoside (structure **20**, Fig. 2) in cranberry

powder (Vvedenskaya et al., 2004). Two of these compounds (structures **17** and **18**, Fig. 2) exhibit a rare structural characteristic—a phenolic acid substituted on a sugar unit of a flavonol-glycoside—and present a new class of phytochemical never before observed in cranberries. In all, this study detected twenty-two distinct compounds believed to be flavonols, fifteen of which were identified. Vvedenskaya and Vorsa (2004) confirmed the presence of all of these flavonols in whole cranberry fruit as well, showing the remarkable diversity and abundance of flavonols in cranberries and cranberry products.

Flavonols are relatively unimportant to cranberry color, primarily contributing yellow undertones. However, these flavonoids are now thought to underlie many cranberry health benefits (Neto, 2007b; Vorsa et al., 2007a). Cranberry flavonols are rather unique in character, containing distributions not found in other fruits, and this in itself makes them very interesting to study. However, it is the unparalleled high concentrations of cranberry flavonols that seem most significant to health effects, which will be discussed further in Sections on the Health Effect of Cranberries, Bioavailability and Metabolism, and Molecular Mechanisms Underlying Health Effects.

Total flavonol contents of cranberries are consistently high across cultivars and high compared to related species such as blueberry and blackberry (Bilyk and Sapers, 1986), ranging from ∼200 to ∼400 mg/kg in fresh cranberries (Zheng and Wang, 2003; Vvedenskaya and Vorsa, 2004/11; Harnly et al., 2006). Indeed, a recent review lists cranberries as the most abundant source of flavonols among thirty plant foods known to contain these bioactive phytochemicals (Aherne and O'Brien, 2002). Yellow onion (350–1200 mg/kg) and curly kale (300–600 mg/kg) have similar or higher levels of total flavonols than cranberries, but no fruits exceeded cranberry flavonol contents by weight (Manach et al., 2004). One recent investigation revealed that flavonol concentrations are remarkably constant over the development of both Ben Lear and Stevens cranberry, ranging from ∼35 to 45 mg/100 unripe to mature. In contrast, anthocyanins increase dramatically from ∼0 to >100 mg/100 g as the fruit ripens, while proanthocyanidins levels start high (180–220 mg/100 g) at berry onset, decrease by about half to 60–110 mg/100 g during early stages of development, and then increase again slightly to 80–130 mg/100 g as the berry ripens (Vvedenskaya and Vorsa, 2004).

Cranberry juice cocktail contains 4.85 mg/100 mL total flavonols (Cunningham et al., 2004), higher than any other beverage reviewed by Aherne et al. (2002) and nearly twice the flavonol content of 12 other commonly consumed, commercially available juices: purple grape, red grape, pomegranate, clear apple, cloudy apple, grapefruit, reconstituted concentrated orange, fresh orange, tropical fruit blend, white grape, pineapple, tomato (Mullen et al., 2007). Interestingly, flavonol concentrations are lower than anthocyanins in ripe cranberry fruit (Vvedenskaya and Vorsa, 2004), but are about three times higher than anthocyanins in cranberry juice (Cunningham et al., 2004; Mullen et al., 2007). Lower anthocyanin levels in juice could be due to several factors, including incomplete anthocyanin extraction during pressing and greater susceptibility to oxidative degradation, as will be discussed further in the Section titled Effects of Processing, Storage and Composition on Cranberry Phytochemical Stability.

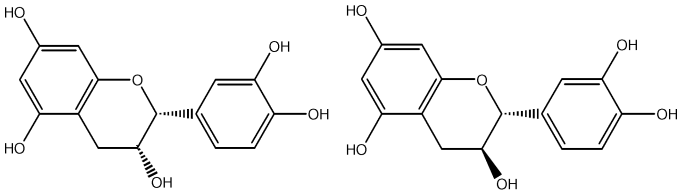


Figure 3 Molecular structures of selected cranberry catechin monomers: the stereoisomers (−) epicatechin (**22**) and (+) catechin (**23**).

Data on individual flavonol contents of cranberries is somewhat more limited (Table 2). Zheng and Wang (2003) report hyperin (7.04 mg/100 g), quercetin (4.16 mg/100 g), myricetin 3-arabinoside (3.75 mg/100 g) and avicularin (3.45 mg/100 g) in Ben Lear cranberries. Harnley et al. (2006) found 16.6 mg myricetin and 19.4 mg quercetin per 100 g hydrolyzed raw cranberries. These are substantial concentrations considering estimates of 20–22 mg/day combined total flavonol and flavonone dietary intake in American doctors (Sampson et al., 2002). Flavonol concentrations in cranberry juice cocktail are somewhat lower due to dilution and perhaps oxidation: 2.32, 1.30, 0.53, 0.52, and 0.18 mg/100 mL respectively for hyperin, quercetin, myricetin, quercitrin, and avicularin (Cunningham et al., 2004).

Catechin Monomers (Flavan-3-ols)

Cranberries contain high levels of flavanols or flavan-3-ols (Fig. 3), which are also monomers of proanthocyanidin polymers (PACs). Contents of individual flavanols in cranberries are reported in Table 2.

Gu et al. (2004) detected 7.3 mg/100 g of total PAC monomer in whole cranberries (variety not identified) and 6 mg/L in cranberry juice cocktail. However, which specific flavan-3-ols are present in cranberries remains controversial, perhaps due to differences among cranberry varieties and analytical methods. Some evidence indicates that epicatechin (structure **22**, Fig. 3) is the primary and perhaps only free flavanol in cranberry juice (Cunningham et al., 2004), while catechin (structure **23**, Fig. 3) was the only flavanol found in commercial cranberry juice cocktail and whole juice (Chen et al., 2001). Recently, gallocatechin gallate (0.4 mg/100 g), catechin (0.8 mg/100 g), epigallocatechin (1.5 mg/100 g), epigallocatechin gallate (1.9 mg/100 g) and larger amounts of catechin gallate (7.9 mg/100 g) have been reported to be present in whole cranberries after hydrolysis (Harnly et al., 2006). However, these identifications must be considered tentative since only HPLC-PDA was used for characterization; whether these gallolyated flavan-3-ols are reaction artifacts must also be determined.

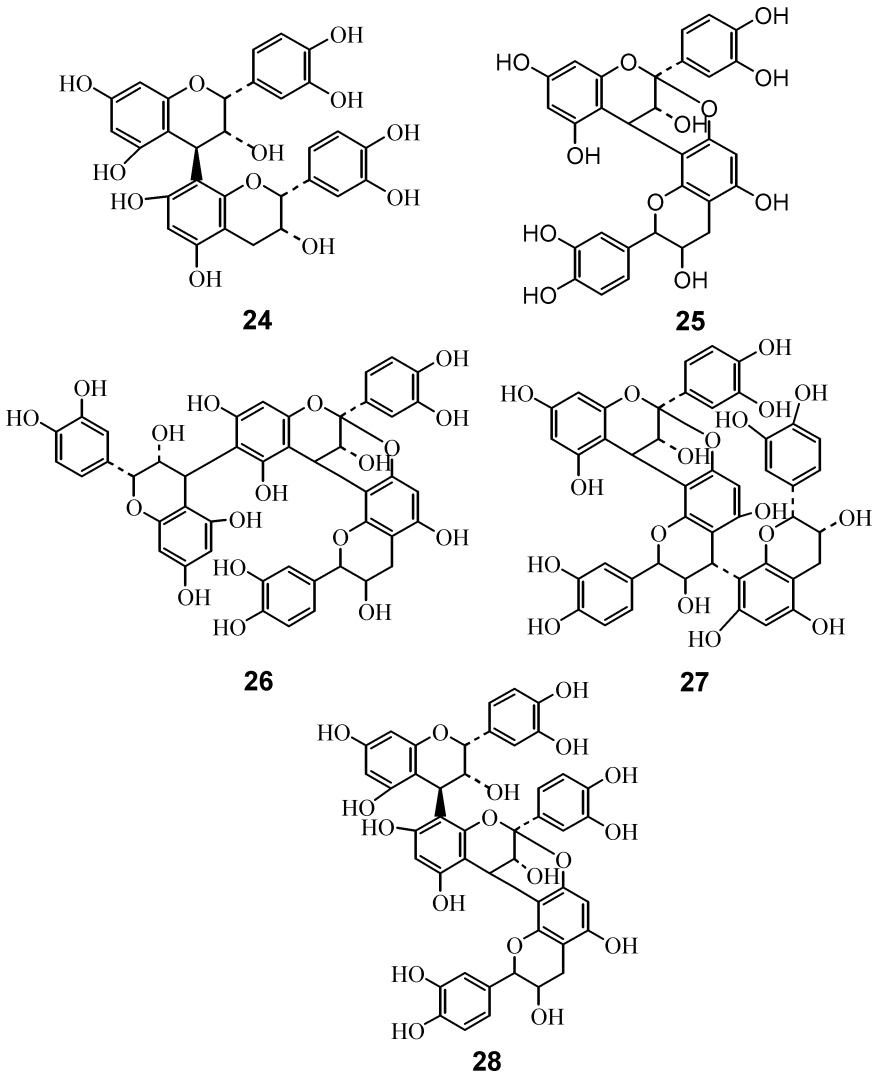


Figure 4 Structures of proanthocyanidins identified in cranberries: procyanidin B2 (EC-(4β→8, 2β→O→7)-EC) (**24**); procyanidin A2 (EC-(4β→8)-EC) (**25**); 3: EC-(4β→6)-EC-(4β→8, 2β→O→7)-EC (**26**); 4: EC-(4β→8, 2β→O→7)EC-(4β→8)-EC (**27**); EC-(4β→8-EC-(4β→8,2 β→O→7)-EC (**28**) where EC = epicatechin.

The total flavanol content of ~7 mg/100 g fruit (Gu et al., 2004) classifies cranberries as a moderate source of flavanols in comparison to other fruits (ranging from 0–20 mg/100 g) (Arts et al., 2000). While the intake of fresh cranberries or canned cranberry sauce may be tied to season or entree, sweetened dried cranberries and cranberry juices have become very popular snacks and beverages eaten in much higher quantities. Considering estimates of 30–70 mg/day total flavanol intake in the United States (Gu et al., 2004) and 50 mg/day in the Netherlands (Arts et al., 2001), cranberries can thus provide a modest but important dietary contribution of these micronutrients.

Proanthocyanidins (PACs)

One unique property of cranberries is their diverse group of proanthocyanidins that exhibit several rare structural characteristics (Fig. 4). Also known as condensed tannins, PACs are defined as oligomers or polymers of flavan-3-ols. Structural characteristics of PACs underlie multiple functions: polyphenolic structures promote antioxidant capacity while vicinal hydroxyl groups bind metals. Perhaps most distinctively, their size, high molecular weight, and many free hydroxyl groups allow PACs to interact with (and often denature or precipitate) proteins. This activity in the saliva is responsible for the astringent or bitter tastes from cranberry, wine, cocoa and other PAC rich foods (Santos-Buelga and Scalbert, 2000). A recent review by Prior and Gu (2005) compiles excellent information about the types, the levels, and the health effects of these bioactive phytochemicals in commonly consumed foods. Although several individual cranberry proanthocyanidin species have been identified, PACs so far have been quantitated only by degree of polymerization and not individually (Table 2), due to their structural heterogeneity and difficult analytical

separation. Gu et al. (2004) report 23.1 mg/100 mL total PAC in cranberry juice cocktail and 418 mg/100 g in fresh cranberries (unspecified varieties). In comparison, among twenty-one fruits evaluated, only chokeberry showed higher PAC contents. The estimated total daily PAC consumption in the US is only 57.7 mg/person (Gu et al., 2004). Thus, even with only small portions, cranberries certainly have the potential to be a major dietary source of these phytochemicals.

Structural diversity and the lack of analytical standards pose significant challenges for PAC research. Cheynier (2005) notes that there are n^x $(n\text{-}1)^y$ possible PACs for an n-mer with x types of constitutive units and y types of linkages. Cranberry PACs contain three constitutive units [epicatechin (EC), epigallocatechin (EGC) and catechin] (Foo et al., 2000a; Gu et al., 2003a; Reed et al., 2005) and at least three types of linkages: two common B-type linkages (C4→C6 and C4→C8) and the relatively uncommon A-type ether linkage (C2→O→C7) (Foo et al., 2000a). Degrees of polymerization as high as 23 have been observed in cranberries (Reed et al., 2005). Applying Cheynier's model to cranberry PACs yields a dramatic figure close to half a billion (483,833,152) different polymers possible. Even though only a miniscule portion of the individual PACs from this model appear to be present in cranberries (e.g. only a handful of the 216 theoretically possible trimers have been observed), their immense heterogeneity remains evident.

Research has only begun to scratch the surface in elucidating structures of individual cranberry PACs, despite intensive research. Isolation of single chemical species and analyses by LC-MS, NMR, and chemical degradation have positively characterized only a few cranberry PACs. These methods have identified two procyanidin dimers: A2 (EC-(4β→8, 2β→O→7)-EC) (structure **24**, Fig. 4) and B2 (EC-(4β→8)-EC) (structure **25**, Fig. 4) as well as three A-type procyanidin trimers: EC-(4β→6)-EC-(4β→8, 2β→Oto7)-EC (structure **26**, Fig. 4), EC-(4β→8, 2β→Oto7)EC-(4β→8)-EC (structure **27**, Fig. 4) and EC-(4β→8)-EC-(4β→8,2β→O→7)-EC (structure **28**, Fig. 4) (Foo et al., 2000b). Even more prevalent are PAC tetramers and pentamers composed of predominantly epicatechin units, with epigallocatechin and catechin extending units, and mostly A-type terminal units in cranberry extracts (Foo et al., 2000a).

Beyond these, characterizations of cranberry PACs have relied almost entirely upon mass spectral techniques and chemical degradation of mixtures of PACs. MALDI-MS currently seems to be the analytical instrument of choice to characterize heterogeneous polymers such as cranberry PACs. While these methods can not fully reveal chemical configurations of individual species, they yield crucial information: DP, type of constitutive units, number of A-type linkages and estimations of the distributions of each. Employing such methodology, several reports have documented multiple procyanidin polymers with DP of 4> 10>, most including one to multiple A-type linkages, but some with only B-type linkages (Porter et al., 2001; Prior et al., 2001; Gu et al., 2002, 2003a, 2004; Howell et al., 2005; Reed et al., 2005; Neto et al., 2006). Epicatechin units are dominant but EGC units are also present (Foo et al., 2000a; Porter et al., 2001; Howell et al., 2005; Reed et al., 2005; Neto et al., 2006); data verifying the presence of catechin units in cranberry PACs is more limited (Foo et al., 2000a; Gu et al., 2003a; Gu et al., 2003a). This apparent discrepancy in findings regarding the constitutive monomer units of cranberry PACs is intriguing and is perhaps due to cultivar variations.

Estimates of average degree of polymerization in cranberry PACs range from 4.7 (Foo et al., 2000a) to 8.5 (Gu et al., 2003a). The latter estimate is likely more accurate since Foo's extraction procedure may exclude larger PACs. A-type linkages dominate in cranberry PACs; an estimated 51–65% of cranberry PACs contain at least one A-type linkage, with most of these ether linkages in the terminal units (Foo et al., 2000a; Gu et al., 2003a). The terminal A-type bonds common to cranberry PACs are quite rare in foods: peanuts and plums are the only other foods containing them, although at substantially lower levels (Gu et al., 2002, 2003a; Prior and Gu, 2005). Similarly, PACs containing both terminal and extension A-type linkages are unique to cranberries and peanuts (again at low concentrations); these were not found in any other of 88 foods tested (Gu et al., 2003a; Gu et al., 2004). While no estimations of average intake are available for A-type PACs, cranberry certainly is among the richest source of these rare phytochemicals in the American diet.

Flavanones

Flavanones have only recently been identified in cranberries. Prunin (naringenin 7-glucoside) was isolated from cranberry juice concentrate in modest amounts (~1.5 ppm) and characterized by NMR (Turner et al., 2005). Although it was found in a cranberry fraction that exhibited anti-adhesive activity towards uropathogenic bacteria, isolated prunin did not exhibit similar activity.

Nonflavonoid Polyphenols

Although largely overshadowed by the flavonoid components, nonflavonoid polyphenols also contribute to the biological effects of cranberries. Perhaps best known of these phenols is the bioactive stilbene, resveratrol. Despite intense publicity on the antioxidant activity of resveratrol in red wines, there has been surprisingly little research on this active compound in cranberries. HPLC-MS identified *trans*-resveratrol (structure **30**, Fig. 5), the isomer with the highest bioactivity, in raw cranberry juice at ~0.2 mg/L (1.91 nmol/g) (Wang et al., 2002); levels were comparable to those found in grape juice (~0.05 to 0.5 mg/L depending on variety) though lower than in red wine (as high as ~14 mg/L) (Baur and Sinclair, 2006). Thus, cranberries can be an important dietary source of this phytochemical.

It is likely that cranberries contain predominantly bound forms of resveratrol. Wang et al. (2002) used β-D-glucosidase

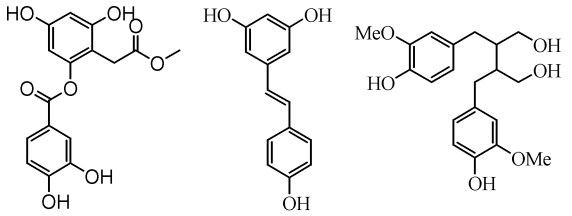


Figure 5 Structures of selected non-flavonoid polyphenols in cranberries: 2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate (**29**); *trans*-resveratrol (**30**) and SECO (**31**).

digestions before extraction, so the *trans*-resveratrol reported probably includes bound forms of the polyphenol, such as *trans*-piceid (resveratrol-3-*O*-β-D-glucoside). Piceid has been identified in grapes, as well as other foods (Baur and Sinclair, 2006) and may exert health effects similar to those of resveratrol, as well as some distinctive ones.

Cis-resveratrol is present in cranberry juice in lower concentrations (0.14 nmol/g juice) (Wang et al., 2002). Total resveratrol levels of 900 ng/g (dry weight) in lyophilized wild cranberries from Canada (Rimando et al., 2004) and 0.19 mg/g (dry weight) in cranberry powder (Vattem et al., 2006) have been reported.

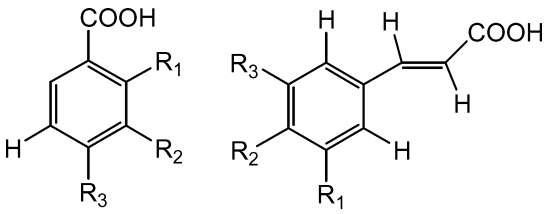
Other phenols identified in cranberry juice concentrate include phloridzin (phloretin 2-glucoside) (Turner et al., 2005), a dihydrochalcone known for its antioxidant capacity and abundantly occurring in apples (Lotito and Frei, 2004), and a novel depside, 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate (structure **29**, Fig. 5) (Turner et al., 2007). Whole cranberry fruit has low concentrations of ellagic acid (120 ug/g dry weight) (Daniel et al., 1989) and the lignan SECO (secoisolariciresinol) (10.54 mg/kg dry weight) (structure **31**, Fig. 5) (Mazur et al., 2000). SECO, a phytoestrogen found mostly in glycoylated forms, required extensive hydrolysis for release from the berry tissue. The levels present in cranberries are intermediate relative to other berries, but high in comparison to other fruits and plant foods.

Additional research is warranted to verify the concentrations of these promising phytochemicals in cranberry products, to determine their variability across cranberry varieties and products, and to investigate their individual health effects.

Simple Phenolics and Benzoates

Simple phenolics and benzoates are important aromatic phytochemicals providing potent antioxidant and antimicrobial activity as well as strong odors and aromas. Benzoates, in particular, are present in cranberries at unusually high levels. Surprisingly, little attention was given to simple phenolics and benzoates of cranberry until recently, despite extensive studies of these compounds in apples, citrus fruits, and other berries (Zuo et al., 2002). Current data on the simple aromatics in cranberry are presented in Table 2; representative structures are shown in Table 4.

Table 4 Structures of selected phenolic and hydroxycinnamic acids from cranberries



Structure #	Name	R_1	R_2	R_3
	Phenolic acids			
32	benzoic acid	H	H	H
33	salicylic acid	OH	H	H
34	2,4-dihydroxy benzoic acid	OH	H	OH
35	vanillic acid	H	OCH_3	OH
	trans-Cinnamic acids			
36	*trans*-Cinnamic acid	H	H	H
37	*p*-coumaric acid	H	OH	H
38	caffeic acid	OH	OH	H
39	ferulic acid	OCH_3	OH	H
40	sinapic acid	OCH_3	OH	OCH_3

Unlike most fruit juices, aromatic compounds rather than terpenes dominate the active odor fraction of cranberry juice. Croteau and Fagerson (1968) identified the following fifteen volatile aromatics in cranberry juice by GC retention times and mass spectrometry:

most abundant – benzoic acid (structure **32**, Table 4)
benzyl alcohol
benzaldehyde
benzyl benzoate
2-phenyl ethanol benzoic acid
lower concentrations – 4-methoxy benzaldehyde
2-phenyl ethanol
dibutyl phthalate
2-hydroxy diphenyl
methyl benzoate
ethyl benzoate
benzyl formate
benzyl ethyl ether
benzene
acetophenone.

Eugenol and anisaldehyde are also present (Duke, 1992).

The soluble phenolic fraction of cranberries contains a large number of compounds that undoubtedly contribute to the antioxidant capacity of cranberries and cranberry products. Low total benzoates ($\ll$0.1% of fresh weight) were initially reported (Marwan and Nagel, 1986). However, more sensitive instrumentation has revealed a much more diverse profile of benzoates and simple phenolics, verified unusually high levels in cranberry juice, and confirmed that phenolics and benzoates

in cranberries are mostly bound forms, esterified to sugars, cell wall polysaccharides, or other components. Less than 50% (most at <10%) of the benzoates and simple phenols are present in their free form (released without hydrolysis) (Zuo et al., 2002). Indeed, hydrolysis is often required for the detection of simple phenols, and the compounds released appear to be specific for the hydrolysis method used, perhaps because they are released from different binding sites. For example, fresh cranberry juice had 41 mg/L benzoic acid in free form, while total benzoic acid (free + bound) after acid hydrolysis increased to 178 mg/L (Chen et al., 2001). Acid hydrolysis also released *p*-anisic acid (2.2 mg/L) in fresh cranberry juice as well as chlorogenic acid (structure **41**, Table 4) (5.1 mg/L) and *p*-coumaric acid (structure **37**, Table 4) (5.2 mg/L) in canned cranberry juice (Chen et al., 2001). Alkaline hydrolysis of cranberry juice cleaved salicylic acid (7.04 mg/L) (Duthie et al., 2005), while enzymatic hydrolysis of cranberry pomace generated gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid as major products (Zheng and Shetty, 2000). This single report of gallic acid monomer in cranberry products is very interesting considering the presence of gallic acid substitutions in cranberry flavanols and proanthocyanidins.

With and without acid hydrolysis, GC-MS analysis found significantly high levels of free and bound benzoic and other phenolic acids (0.57% wet weight) in fresh cranberries (Zuo et al., 2002). Benzoic acid (0.47%) accounted for the majority of this fraction, with lesser amounts of fourteen other phenolic and benzoic acids (Zuo et al., 2002) (Table 4):

Bound – *o*-hydroxybenzoic acid (salicylic acid) (structure **33**, Table 4)
p-hydroxyphenylacetic acid
2,3-dihydroxybenzoic acid
hydroxycinnamic acids
Free – *trans*-cinnamic acid (structure **36**, Table 4)
m-hydroxybenzoic acid
p-hydroxybenzoic acid
o-phthalic acid, vanillic acid (structure **35**, Table 4)
2,4-dihydroxybenzoic acid (structure **34**, Table 4).

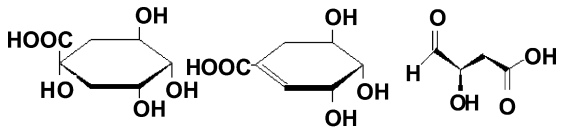
Of hydroxycinnamic acids, *p*-coumaric acid and sinapic acid (structure **40**, Table 4) are most prevalent, but significant amounts of caffeic acid (structure **38**, Table 4) and ferulic acid (structure **39**, Table 4) are also present.

The molecular sites of phenol binding in cells appear to be mostly saccharides, but the preference and distribution between low molecular weight glycosides and more complex polysaccharides is not yet clear. Most phenols appear to be bound to low molecular weight species, e.g. linked to mono and disaccharides by ether or ester bonds. Complexed forms identified in cranberry extracts or juice concentrates include hydroxycinnamic acid esterified to ursolic acid (Murphy et al., 2003; He and Liu, 2006), coumaric acid bound to iridoid glycosides (Turner et al., 2007), benzoic acid and hydroxycinnamic acid esterified to mono- and di-saccharides (Marwan and Nagel, 1982, 1986; Heimhuber et al., 1990; He and Liu, 2006; Mullen et al., 2007), and glycosylated derivatives of *p*-coumaric, caffeic, ferulic and sinapic acids (Marwan and Nagel, 1982), vacciniin (6-benzoyl-D-glucose) (Marwan and Nagel, 1986), and three additional glycosylated benzoic acid isomers (Heimhuber et al., 1990). Vvedenskaya et al. (2004) similarly isolated and characterized simple phenolic compounds bound to several flavonol glycosides in cranberry powder. All these newly identified compounds are rare and several appear unique to cranberries.

Other work suggests a significant proportion of cranberry phenolics are bound to complex carbohydrates or other macromolecules. Zhang and Shetty (2000) used pure β-D-glucosidase as well as crude enzymatic extracts from *L. edodes* to release phenolics from cranberry pomace, which is composed of mostly insoluble fiber. That the crude enzyme containing an esterase (α-L-arabinofuranosidase, α-L-rhamnosidase, and/or β-D-apiosidase) produced notably higher yields of phenolics than did pure β-D-glucosidase suggests that most of the phenolics in cranberry pomace are bound to more complex carbohydrates and/or more diverse chemical species, potentially including substituted proanthocyanidins (PACs). These complex carbohydrate-phenolic acid esters and substituted PACs are heterogeneous in structures and linkages, making it difficult to isolate individual species for characterization. Still, the complexes may be present in the high molecular weight fraction of cranberry juice that has been the subject of significant research (Section titled High Molecular Weight Fraction (NDM)), and they also are likely to have some biological activity, especially as antioxidants. Further research is needed to fully elucidate the nature and health effects of low molecular weight bound phenolics from cranberries.

Nonphenolic Organic Acids

Nonphenolic organic acids (Table 2 and Fig. 6) are significant components of cranberries, accounting for 2.67–3.65% w/v of the berry tissue (Jensen et al., 2002) and ~2.75% w/w of whole cranberry juice (Hong and Wrolstad, 1986a). The three primary nonvolatile organic acids—quinic acid (structure **41**, Fig. 6), citric acid, and malic acid of cranberry juice (Coppola et al., 1978; Coppola and Starr, 1986; Hong and Wrolstad, 1986a) are present in very consistent ratios (~1:1:0.75, respectively).



45 46 47

Figure 6 Structures of selected non-volatile organic acids from cranberries: quinic acid (**41**), shkimic acid (**42**) and the methyl ester of tartaric acid (**43**).

These ratios together with the uniquely high content of quinic acid in cranberries form the basis for the HPLC-UV assay used extensively in the cranberry juice industry today to detect the adulteration in cranberry juice products.

Ascorbic acid (vitamin C) has many reported health benefits as an antioxidant and nutrient and its dietary deficiency is widely known to cause scurvy. Vitamin C is present in whole cranberries at concentrations that are moderate (~11.5 mg/100 g) (Licciardello et al., 1952) but sufficient to prevent scurvy among early American settlers (Henig and Leahy, 2000). Ascorbic acid levels in cranberry juice cocktail and sauce are lower (2–4 mg and 1 mg/100 g, respectively) (Licciardello et al., 1952; Cunningham et al., 2004; Starr and Francis, 1968) or absent (Hong and Wrolstad, 1986a). Hence, cranberry products are often fortified with vitamin C to meet consumer expectations.

Lower levels of shikimic acid (0.1–0.9 g/100 ml) (structure **42**, Fig. 6) (Hong and Wrolstad, 1986a) and galacturonic acid (0.19 g/100 ml) (Cunningham et al., 2004) as well as trace amounts of tartaric acid and its methyl ester (structure **43**, Fig. 6) (He and Liu, 2006) have been found in whole cranberry juice. Galacturonic acid is thought to accumulate from commercial enzymatic depectinization (Cunningham et al., 2004). These acids have been well characterized and quantified by ^{1}H and ^{13}C NMR and MS (He and Liu, 2006).

Cranberry juice cocktail is generally standardized to ~0.5% titratable acidity. It is this high acidity, similar to that of lemon, as well as the low content of natural sugars (Section titled Sugars) that demands the sweetening of cranberry products to reduce astringency and enhance natural flavors. While vital to flavor, organic acids other than vitamin C are not known to have important health effects.

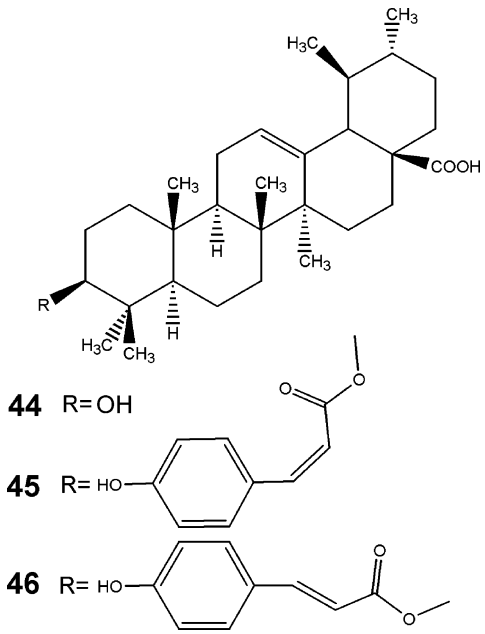


Figure 7 Ursolic acid (**44**) and its derivatives, *cis*- and *trans*- 3-*O*-*p*-hydroxy cinnamoyl ursolic acid (**45 and 46**), found in cranberries.

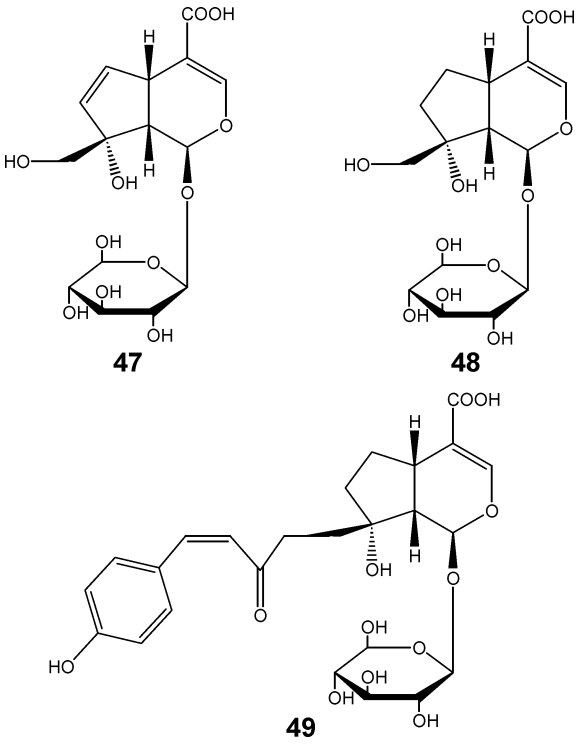


Figure 8 Iriodoid glycosides from cranberries: monotropein (**47**), 6,7 dihydromonotropein (**48**) and 10-*p*-*cis*-coumaroyl-1*S*-dihydromonotropein (**48**).

Terpenes, Sterols, and Terpene Derivatives

Terpenes and terpene derivatives such as nerol, limonene, linalool, myrcene, α-pinene, β-pinene and especially α-terpineol are responsible for much of the flavor and aroma of cranberries (Croteau and Fagerson, 1968). Table 2 lists the component terpenes of cranberries, and their quantities, when known. Figures 7 and 8 shows selected terpene molecular structures. These odorants are common in foods and are used extensively as food flavoring additives, so do not likely elicit the health effects unique to cranberry.

Ursolic acid (structure **44**, Fig. 7), a pentacyclic triterpene, identified in cranberries more than half a century ago (Wu and Parks, 1956), has more recently attracted attention for its potential anticancer effects (He and Liu, 2006). Two rare hydroxycinnamic derivatives of this triterpene were also recently isolated by HPLC and identified by MS as well as ^{1}H and ^{13}C NMR in cranberry: *cis*- and *trans*- 3-*O*-*p*-hydroxy cinnamoyl ursolic acid (structures **45** and **46**, Fig. 7) (Murphy et al., 2003; He and Liu, 2006). Recently, 60–110 mg/100 g ursolic acid was quantified in whole cranberries of different cultivars (Kondo, 2006).

He and Liu (2006) also isolated two sterols, β-sitosterol, and β-sitosterol-3-*O*-β-D-glucoside, from whole cranberry fruit using bioactivity guided fractionation and identified the structures by MS and ^{1}H and ^{13}C NMR. These compounds are widely distributed in plants but may nonetheless contribute subtly to cranberry health effects (He and Liu, 2006).

Another study revealed the presence of two related iridoid glycosides: monotropein (structure **47**, Fig. 8), and 6,7-dihydromonotropein (structure **48**, Fig. 8) by HPLC-NMR and HPLC-MS (Jensen et al., 2002). The isolated amounts correspond to about 0.01% w/v each in whole cranberry juice. Two isomeric derivatives of 6,7-dihydromonotropein [10-*p-cis*-(structure **49**, Fig. 8) and 10-*p-trans*-coumaroyl-1*S*-dihydromonotropein] have been recently isolated and characterized from cranberry juice concentrate (Turner et al., 2007). Aside from monotropein, these iridoid glycosides are novel and may be unique to cranberry, although they have not been linked to health effects (Jensen et al., 2002; Turner et al., 2007).

The terpene fraction of cranberry has only very recently attracted considerable research attention beyond its odor contributions, and several exotic chemical species have been identified. Future research will reveal whether or not these interesting phytochemicals contribute to the health benefits of cranberries.

Complex Carbohydrates

Consumption of complex carbohydrate fibers has been associated with a number of health benefits, none of which are typical of cranberries. Because of this, or perhaps because cranberry juice (with little fiber) is the major form of cranberry consumption, complex carbohydrates of cranberries have been largely ignored. Cranberry pomace solids are 35% insoluble fiber (USDA-ARS, 2004), the pectins of which are largely responsible for cranberry sauce gelation, and more extensive utilization of fiber by-products has recently been the focus of some research (Zheng and Shetty, 1998, 2000; Park and Zhao, 2006; Raghavan and Richards, 2007).

Not surprisingly, an early investigation into the cell wall composition of cranberries revealed the presence of cellulose, pectin, and hemicellulose in cranberry pomace (Holmes and Rha, 1978). The alcohol insoluble fraction of lyophilized berries is mostly protopectin (54–67%, with lower levels of high methoxy pectin (10–18%); overall degree of esterification in the pectins is 47.7–57.5% depending on variety (Stuckrath et al., 1998). The soluble fiber fraction of cranberries contains monomer units mostly of arabinose, glucose and galactose/rhamnose, with lesser amounts of xylose and mannose (30, 28, 21, 11, and 10% respectively) (Marlett and Vollendorf, 1994).

Although whole cranberry juice, like most juices, contains very little fiber (0.1%) (Cunningham et al., 2004), cranberry sauce contains substantial levels, ∼1%, of these insoluble carbohydrates (Marlett and Vollendorf, 1994). Interesting data suggesting covalent interactions between complex carbohydrates and phenolics in cranberries (Zheng and Shetty, 2000) was discussed above (Section titled Phenolic Acids and Benzoates). Such interactions may improve the potential health benefits of these fibers as well as provide functionality to products derived from them. Indeed, edible films with unique properties, such as natural bright color and antioxidant activity, have been developed from cranberry pomace (Park and Zhao, 2006).

High Molecular Weight Fraction (NDM)

Numerous studies have reported substantial health effects from the high molecular weight fraction of cranberry, known as cranberry nondialyzable material or NDM (Zafriri et al., 1989; Ofek et al., 1991; Ofek et al., 1996; Weiss et al., 1998; Burger et al., 2002; Shmuely et al., 2004; Shmuely et al., 2007; Weiss et al., 2004; Steinberg et al., 2005; Weiss et al., 2005; Steinberg et al., 2005; Bodet et al., 2006a, 2006b; Labrecque et al., 2006; Bodet et al., 2007a). NDM is prepared by dialysis of fresh cranberry juice or cranberry juice concentrate with MW cut off of 12,000–15,000, often at refrigerated temperatures. Ofek et al. (1996) described NDM as tannic in nature; soluble in water; free of proteins, carbohydrates, and fatty acids; and composed of 4.14% hydrogen and 56.6% carbon. Others found that NDM contains no sugars or acids but consists of 0.35% ACY and 65.1% PAC (Bodet et al., 2006a; Bodet et al., 2006b). It seems likely that cranberry NDM is a heterogeneous mixture of condensed phenols and flavonoids whose structural variability will prevent any concrete structural elucidation. Whether this elusive fraction is naturally in cranberries or results from processing is also unknown. Further characterization is needed to more fully understand its observed activities.

HEALTH EFFECTS OF CRANBERRIES, THEIR PRODUCTS, AND COMPONENTS

Numerous health benefits have been associated with cranberry consumption. Unique to cranberries among foods are antipathogenic effects including:

- Prevention of urinary tract infections
- Clearance of stomach pathogens
- Disruption of oral pathogen virulence and biofilm formation
- Depression of viral infectivity in vitro

Cranberries have also been shown to inhibit progression of degenerative disease and loss of functionality, with the following health benefits:

- Inhibition of Alzheimer's disease development and neural degeneration
- Promotion of cardiovascular health
- Prevention and inhibition of cancer
- Modulation of inflammatory responses

Scientific evidence supporting these health effects varies greatly. The following sections will provide brief overviews of each disease state and discuss observations from epidemiological, in vitro, ex vivo, and in vivo data affirming or refuting the reported health benefits. Special emphasis will be given to the specific phytochemicals implicated in each action. Molecular

mechanisms will be considered in Section titled Molecular Mechanisms Underlying Health Effects.

Urinary Tract Infections

Best-known and most extensively documented of cranberry health effects is prevention of urinary tract infections (UTIs). Occurring primarily in women, UTIs are surprisingly common and quite costly. An estimated 11.3 million women (about one in ten women) in the United States per year contract infections, at a cost of $1.6 billion annually (Foxman et al., 2000). Even more troublesome, UTIs are often recurring: 26.6% of women with a UTI were confirmed to have a second infection within 6 months (Foxman, 1990).

Cranberries have long been a folk remedy for UTIs, and science is now providing both verification of cranberry efficacy and explanations for cranberry actions. Clinical trials have convincingly demonstrated reduced bacteriuria and/or lowered occurrence of UTIs in groups of women consuming cranberry products including juices, extracts, and powders on a daily basis for at least several weeks (Avorn et al., 1994; Walker et al., 1997; Kontiokari et al., 2001; Stothers, 2002; Bailey et al., 2007). A recent meta-analysis of clinical data revealed a relative risk of 0.65 for symptomatic UTIs and 0.61 for recurrent UTIs for subjects consuming cranberry products as compared to control or placebo groups (Jepson and Craig, 2007). A case-control study of 324 adult women with and without previous UTIs found that those who consumed more fruit juice, berry juices in particular, faced significantly lowered risk of contracting UTIs. Odds ratios (OR)[1] were reduced from 1 (equal incidence) to 0.66 ($p < 0.011$) after consumption of just over 200 mL juice per day, and the effect was enhanced even further ($OR = 0.28$, $p < 0.001$) among subjects who preferred berry juices (including cranberry, lingonberry, blueberry, raspberry, strawberry, currant, and cloudberry) over other juices (Kontiokari et al., 2003). Within this group, even occasional cranberry consumption (<1 serving per week) was associated with lowered risk of UTI ($OR = 0.48$, $p < 0.03$). Statistical analysis of regular consumption of cranberry was not possible because none of the 139 women in the study with a recent UTI had consumed more than one serving of cranberry juice per week.

UTI effects of cranberries appear to be specific for or most pronounced in women. Groups at increased risk for contracting UTIs, including children, the elderly, and patients needing intermittent catheterization, have shown little or no benefit from cranberry product supplementation (Avorn et al., 1994; Haverkorn and Mandigers, 1994; Foda et al., 1995; Schlager et al., 1999; Linsenmeyer et al., 2004; Waites et al., 2004; McMurdo et al., 2005; Jepson and Craig, 2007). In addition, cranberry effects against UTI appear to be exclusively prophylactic but not therapeutic, preventing the development of infection but inactive against existing UTIs.

How do cranberries prevent development of UTI's? *Escherichia coli* is the pathogen responsible for the most UTIs, accounting for 75–95% of infections (Gupta et al., 2001; Jepson and Craig, 2007). The use of antibiotics to treat recurrent infections increases pathogen resistance to them, so that resistant strains now account for 7–60% of infections (Gupta et al., 2001; Jepson and Craig, 2007). As currently understood, UTIs begin in the periurethral tissue where bacteria colonize. Protein-based adhesins or lectins expressed on the surface of these bacteria bind to glycoproteins and/or glycolipids on host cell surfaces, allowing for subsequent colonization (Sharon and Ofek, 2002) in the urinary tract and finally in the bladder (Jepson and Craig, 2007). In the case of uropathogenic *E. coli*, these lectins have been identified as primarily mannose binding type-1 adhesins and in some strains, additionally, the galabiose-binding P-fimbriated adhesins (Beachey, 1981). This pathogen-host cell interaction is essential to infection, since innate cleansing mechanisms such as urine flow will otherwise flush pathogens from the bladder and urinary tract. In the face of increasing antibiotic resistance, the failure of antibiotic treatments to prevent recurring infections, and high costs associated with UTIs, alternative therapies like supplementation of cranberry products are increasingly attractive.

Until recently, anti-UTI action was ascribed to cranberry's high acid content and acidification of urine. Sobota (1984) then found that when mice and humans were fed cranberry juice cocktail, their urine inhibited adherence of diverse strains of clinically isolated *E. coli* to uroepithelial cells ex vivo. This ex vivo action has been verified and strikingly demonstrated to be dose-dependent in a placebo-controlled, double-blind clinical trial (Di Martino et al., 2006): as little as 250 mL daily intake of cranberry juice cocktail inhibited adhesion to uroepithelial cells by 45% ($p < 4.9 \times 10^{-12}$), while 750 mL reduced adhesion by 63% ($p < 2.6 \times 10^{-18}$). Exposing uroepithelial cells to cranberry juice directly in vitro inhibited adhesion of E. coli expressing both 1-type and P-type fimbriae, but not another adhesin from a diarrheal isolate (Zafriri et al., 1989; Ofek et al., 1991). (Zafriri et al., 1989; Ofek et al., 1991). The action occurs also in vivo, as demonstrated by reduced bacteriuria observed in women consuming cranberry products (Avorn et al., 1994).

That prevention of bacterial adhesion to epithelial cells in the urinary tract is a major action of cranberries now seems clear. However, many questions about cranberry anti-UTI activity remain unanswered. There is considerable controversy over which components in cranberry juice are actually responsible for the anti-adhesion activity. Early studies attributed activity to fructose for the 1-type adhesin and the high molecular weight NDM fraction for the P-fimbriated adhesin because of their in vitro activity (Zafriri et al., 1989). Recently attention has shifted to proanthocyanidins. Lower molecular weight A-type cranberry PACs were reported to inhibit binding of P-fimbriated (but not

[1]Odds ratios were calculated from comparison of regression curves relating various dietary fators to UTI incidence for subjects with previous UTIs compared to paired sunjects without previous UTIs. For specific fruit comparisons, an OR of 1 was the risk assigned to subjects who never consumed the fruit.

1-type) *E. coli* in vitro (Howell et al., 1998; Foo et al., 2000a, 2000b; Howell et al., 2005; Howell, 2007).

Inconsistency of in vitro and ex vivo data with bioavailability of cranberry phytochemicals poses a serious limitation to elucidating mechanisms. While fructose is active in vitro and is absorbed, in vivo it is preferentially utilized in other metabolic pathways. Indeed, feeding of fructose-rich apple and grape juices failed to promote anti-adhesion properties in urine ex vivo (Howell et al., 2005). After cranberry consumption, collected urine clearly inhibits adhesion of both 1-type and P-type *E. coli* ex vivo (Sobota, 1984; Di Martino et al., 2006). However, in vitro NDM is clearly inactive against 1-type *E. coli* (Zafriri et al., 1989) and PAC activity has not been reported. In inhibiting adhesion of P-type *E. coli*, isolated individual cranberry PACs were active at as low as 0.3 mg/mL and mixed cranberry PACs were active at as low as 75 μg/mL in vitro (Foo et al., 2000a; Foo et al., 2000b). High ppm concentrations such as these, if absorbed, should easily be detected with modern analytical technology. Nevertheless, these components have not been isolated (in any amount) in urine after consumption of cranberry products (see Section titled Bioavailability and Metabolism of Cranberry Phytochemicals), casting great doubt on their activity against UTIs in vivo (Valentova et al., 2007; Vorsa et al., 2007a). Thus, cranberry components active against UTIs in vivo remain a mystery, and are perhaps unknown metabolites of PACs (Howell, 2007) or other, yet to be identified, components.

Even though anti-UTI effects may be limited to women, cranberry foods remain unique as the only nutritional therapies clearly demonstrated to be effective in preventing an infectious disease (Donabedian, 2006). The UTI-cranberry association is so strong that the French health agency AFSSA has recently allowed certain cranberry juices, powders, and concentrates to be marketed as urinary health promoters—the first instance of a government sponsored, specific health claim allowed for an individual fruit.

Much must still be learned about the molecular chemistry underlying gender specificity, inhibition of bacterial adhesion, and other potential mechanisms by which cranberry components prevent urinary tract infections. Direct molecular mechanisms are proposed in the Section titled Molecular Mechanisms Underlying Health Effects, but none of these has yet been proven. Continuing studies are needed to answer these fundamental questions as well as identify adequate minimum dosage required and minimum duration of supplementation for effective treatment.

Gastrointestinal Disease

Accumulating evidence indicates that cranberries also prevent adhesion of the stomach pathogen *Helicobacter pylori* (Burger et al., 2000, 2002; Shmuely et al., 2004; Shmuely et al., 2007; Lin et al., 2005; Vattem et al., 2005). Stomach ulcers and cancers were traditionally attributed to noninfectious mechanisms because the stomach is highly acidic and thus hostile environment to bacteria. However, *H. pylori* have recently been implicated as the primary cause of these diseases. *H. pylori* is ubiquitous, with incidence as high as 90% in some populations; it is now estimated to cause 60–90% of all gastric cancers (Malfertheiner et al., 2005). In 2005 B.J. Marshall and J.R. Warren were awarded the Nobel Prize in Medicine "for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease."

Like uropathogenic *E. coli*, *H. pylori* attach to host cells via adhesions expressed on the bacteria's surface. Many such adhesions have been identified in *H. pylori*, where they facilitate binding to gastric mucus and erythrocytes as well as epithelial cells (Burger et al., 2000, 2002). While the gastric mucus layer is its most common habitat, it is believed that *H. pylori* must penetrate that mucus layer over multiple generations and bind to the epithelial cells to cause gastritis and cancer (Burger et al., 2000). Conventional treatment with two antibiotics and a proton pump inhibitor (amoxicillin, clarithromycin, omeprazole, respectively) is about 85% effective (Shmuely et al., 2007). Higher eradication rates are desired, but antibiotic resistance is already a serious concern and higher antibiotic levels would only increase this risk (Dunn et al., 1997).

Observations that cranberry components inhibit *H. pylori* adhesion to gastric mucosal cells in vitro offer promise that cranberries may prevent the disease altogether or at least reduce the antibiotic levels required to control it. Cranberry NDM were first shown to prevent adhesion of *H. pylori* to gastric mucosal constituents, gastric cell lines, and erythrocytes (Burger et al., 2000, 2002); sialic acid-sensitive lectins binding cells to the mucosa were the key targets. In a subsequent study, low concentrations of cranberry NDM (0.2 mg/mL) inhibited the adherence of 53 of 83 clinically isolated strains of *H. pylori* to a human gastric cell line, indicating that cranberry components must affect multiple but perhaps not all of the adhesions expressed by *H. pylori*. Importantly, NDM inhibited a significantly higher percentage of strains (64%) than did the antibiotic metronidazole (58%) (Shmuely et al., 2004). Synergy between oregano and cranberry constituents in inhibiting these bacteria has also been demonstrated (Lin et al., 2005). Recently, promising clinical evidence has emerged indicating that cranberries are also highly effective in preventing adhesion of *H. pylori* in vivo and thus may inhibit critical steps in the pathogen's virulence therapeutically. In a randomized, double-blind, placebo-controlled trial involving 189 adults infected with *H. pylori*, daily consumption of 500 mL of cranberry juice cocktail resulted in a modest (14%) though significant ($p < 0.05$) decrease in infection relative to placebo (5%) after 35 days, and the effect persisted through the 90 day study (Zhang et al., 2005). These results are unprecedented in demonstrating that consumption of a food was effective in treating an existing pathogenic infection in vivo, not just preventing it.

Gender specificity such as observed with UTI may also be active for *H. pylori* inhibition. When cranberry supplementation was combined with conventional drug cocktail therapy, female patients, but not males, showed higher rates of *H. pylori* eradication (Shmuely et al., 2007).

Despite some notable advances, *H. pylori's* mechanism of infection and transmission remains poorly understood (Zhang et al., 2005). One literature review suggests berry juices and/or probiotic drinks may be effective, low cost treatments in controlling *H. pylori* (Gotteland et al., 2006). Perhaps most importantly, cranberry remains extremely promising as a means to control strains of *H. pylori* resistant to antibiotic therapies. However, more research is needed to verify and clarify a number of critical issues: potential gender specificity, whether dietary supplementation with cranberry products can prophylactically prevent development of ulcers, how cranberries affect interaction of microbial cells with stomach tissues, whether cranberries present a viable alternative to antibiotic treatment alone, or if antibiotics and cranberry supplementation (perhaps along with probiotic therapies) can be used in conjunction to increase therapeutic efficiency.

Dental Health and Gum Disease

Cranberry products and components may reduce bacterial infections in the mouth and thus prevent dental caries and periodontal disease. Bacteria bind to teeth, then aggregate, and cooperating oral bacteria secrete dental plaque biofilms. These biofilms harbor the diverse oral pathogens that produce acid, causing dental caries and eliciting inflammatory immune reactions responsible for periodontal disease. Biofilms create a habitat enabling bacterial reproduction in an environment providing protection from cleansing mechanisms. While *Streptococcus mutans* and *Streptococcus sobrinus* are acknowledged as the primary pathogens responsible for dental caries and *Porphyromonas gingivalis* is the primary pathogen in periodontal disease, several hundred species of bacteria have been found to inhabit the human mouth and may contribute to (or inhibit) these disease states (Liljemark and Bloomquist, 1996). Cranberries and products derived from them may play important roles in limiting plaque formation, dental caries, and periodontal disease.

The first report investigating cranberry effects on dental health revealed that both isolated cranberry NDM and cranberry juice (but not apple juice) prevented and reversed interspecies co-aggregation of bacteria in vitro (Weiss et al., 1998). 2.5 mg/mL Cranberry NDM (approximately the concentration in cranberry juice cocktail) inhibited co-aggregation in 58% of the 84 oral bacterial pairs tested, suggesting that cranberry components may prevent dental disease. These results were later confirmed in a saliva matrix (Weiss et al., 2002). Proliferation of the periodontal pathogens *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola* and their proteolytic virulence factors were inhibited by cranberry NDM in vitro (Bodet et al., 2006b). Cranberry components also prevent biofilm formation, adhesion of *S. Sobrinus*, *S. mutans, P. gingivalis* to tooth-like and oral cell surfaces, and acid production in *S. mutans* (Steinberg et al., 2005; Duarte et al., 2006; Koo et al., 2006; Labrecque et al., 2006). In suppressing *S. mutans* acid production and biofilm formation, cranberry PACs were most active, flavonol effects were intermediate, and ACYs were inactive (Duarte et al., 2006). Inflammation responses stimulated by isolated lipopolysaccharide were also dose-dependently inhibited by cranberry NDM in a model of periodontitis. Thus, cranberry components appear to alleviate gum disease by host interactions as well as the previously discussed pathogen interactions (Bodet et al., 2007a). The cause of tissue damage in gingivitis inflammation will be discussed further in Section titled Anti-Inflammation.

In vivo evidence for cranberry contributions to dental health is very limited. In a preliminary clinical trial, mouthwashes containing NDM and used twice a day for 6 weeks lowered bacterial counts of *Streptococcus mutans* by two orders of magnitude over a control mouthwash (Weiss et al., 2002). While bioavailability of compounds is not a limitation to activity in the mouth, collection of more in vivo data seems justified, especially because consumption of cranberry products is much different than using mouthwash. Clinical trials evaluating the effect of cranberry juice supplementation on patients with periodontitis or high incidence of dental carries may yield very interesting results.

It must be stressed that cranberry juice cocktail, if sweetened with sugar, is not recommended for dental health promotion since the sugar content facilitates rather than prevents dental carries (Weiss et al., 1998). Instead, unsweetened or artificially sweetened cranberry products or isolated cranberry components may provide effective therapies for dental health improvement. Toothpastes supplemented with cranberry phytochemicals (combinations of NDM, PACs and flavonols) as well as mouthwashes consisting entirely of whole cranberry juice or solutions fortified with cranberry components also seem promising as dental health promoters.

Antivirus Activity

That cranberries may significantly reduce infectivity of viral pathogens has only recently been recognized, so little data detailing the action is yet available. Viruses are responsible for some of the most innocuous infections, like the common cold, to the most deadly, notably AIDS, so developing therapies against viral pathogens is critically important. Currently available antiviral therapies are costly and only partially effective. Investigation of alternative therapies such as cranberry supplementation, is clearly needed and may even provide greater insight into mechanisms of infectivity and identification of potential treatment targets.

In vitro cranberry NDM and isolated cranberry PACs inhibited adhesion and infectivity of influenza virus A (subtypes H_1N_1 and H_3N_2) and influenza virus B to human red blood cells (Weiss et al., 2005). NDM activity was ~5 times more potent on a molar basis than isolated PACs (Weiss et al., 2005). In a study of effects of three commercially available fruit juices on the infectivity of several bacteriophages (A2 and A4) and a simian rotovirus (SA-11), orange juice and grapefruit juice reduced the

infectivity of the bacteriophages on *E. coli* by 20–35% over 60 minutes incubation time, but cranberry juice cocktail caused 90–100% reductions in infectivity almost immediately (Lipson et al., 2007a). After treatment with cranberry juice, Simian rotovirus did not infect African green monkey cells, in contrast to the control, and hemagglutination was completely inhibited (Lipson et al., 2007b). In monkey epithelial cell lines treated with isolated cranberry phytochemicals, 25 to 2000 μg/mL isolated proanthocyanidins and flavonols dose-dependently reduced infectivity of bovine reovirus after 3–5 minutes of incubation time. High MW proanthocyanidins were more effective than low MW proanthocyanidins; flavonols and cranberry juice cocktail were mildly active and anthocyanins inactive (Lipson et al., 2007b).

These observations demonstrate a nonspecific antiviral effect exerted by cranberry juice and cranberry components (Lipson et al., 2007a; Lipson et al., 2007b). Interestingly, anti-HIV activity has been documented from several, rare plant flavonoids (flavonols, flavonol glycosides, as well as prenylated and galloylated flavonols) from exotic sources such as the bulbs of the Chinese flower *Chrysanthemum morifolium*, the medicinal Korean herb *Acer okamotoanum*, and leaves of the African rainforest tree *Monotes africanus* (Hu et al., 1994; Kim et al., 1998; Meragelman et al., 2001). Inhibition of the enzyme HIV integrase, vital to HIV's ability to penetrate host cells, has been proposed as a possible mechanism for anti-HIV activity of flavonoids (Kim et al., 1998). Whether similar interference with viral enzymes underlies the anti-viral properties of cranberries is unknown, but some observations support the hypothesis that cranberry components inhibit viral penetration of host cells (Lipson et al., 2007a, 2007b). Viral RNA was not found within cells incubated with cranberry juice and then challenged with bovine reovirus (Lipson et al., 2007b), and electron microscopy failed to detect intact Simian rotovirus in exposed MA-104 cultures after incubation with cranberry juice (Lipson et al., 2007a).

Antiviral activity of cranberry products and components in vivo has not yet been documented. Nevertheless, a greater understanding of the mechanisms involved could have tremendous medical implications since available anti-viral therapies are quite limited.

Cardiovascular Health

Cranberry consumption appears to modulate several biomarkers of cardiovascular health and thus may reduce the incidence of cardiovascular disease, similarly to moderate wine consumption. A comprehensive discussion of the role of cranberries in cardiovascular health is available in a recent review by Ruel and Couillard (2007). Briefly, atherosclerosis is believed to be initiated by the gradual incorporation of oxidized lipoproteins into blood vessel walls, creating fatty lesions. Crucially, surrounding endothelial cells then induce pro-inflammatory responses, including the expression of adhesion molecules (see Section titled Anti-Inflammation), that recruit lymphocytes to the sub-epithelial intima. These immune cells ingest the fatty deposits, which may be cleared or, in the case of atherosclerosis, eventually become encased in hardened plaques that restrict blood flow, resulting in hypertension and damage to the heart. Even worse, they promote blood clotting (thrombosis) or rupture, which may completely halt blood flow, resulting in heart attack or stroke (Neto, 2007a; Ruel and Couillard, 2007).

Epidemiological data suggest a strong inverse correlation between cardiovascular disease and consumption of fruits, vegetables, and flavanoid compounds (Hertog et al., 1993, 1995). Flavonol intake, in particular, has been strongly associated with lower rates of coronary disease mortality and stroke incidence (Hollman and Katan, 1997, 1999). The consumption of cranberries, a fruit with high flavonoid and especially flavonol content, may then promote cardiovascular health, though this effect has not been studied directly epidemiologically.

Oxidation of blood low density lipid protein cholesterol (LDL) is believed to be a crucial step in the early development of atherosclerosis. Moreover, high levels of LDL and low levels of high density lipoprotein (HDL) are widely considered strong risk factors for cardiovascular disease. That cranberries and cranberry components inhibit LDL oxidation in vitro is not surprising since so many cranberry phytochemicals are strong antioxidants (Wilson et al., 1998; Porter et al., 2001; Vinson et al., 2001; Chu and Liu, 2005).

Whether dietary consumption of cranberries is beneficial to overall cardiovascular health is more controversial. Much in vivo data show that cranberry consumption has antioxidant action important in combating cardiovascular disease. After a single 500 mL administration, cranberry juice cocktail (but not a control drink or blueberry juice) significantly raised the plasma antioxidant capacity ($P < 0.001$) as measured by both the ferric reducing antioxidant potential (FRAP) assay and the Fremy's salt radical reduction assay in female subjects (Pedersen et al., 2000). Similarly, plasma antioxidant capacity increased after daily administration of 660 mL of cranberry juice to male and female volunteers for ten weeks: thiobarbituric acid reactive substances (TBARS) in plasma were reduced significantly ($P < 0.05$) from 3.56 (baseline) to 2.08 μM and oxidative lag time of harvested LDL ex vivo was significantly ($P < 0.002$) increased from 50.67 (baseline) to 60.67 minutes (Lu and Wang, 2006). In contrast, the placebo group, who drank simulated cranberry juice with the same level of vitamin C, exhibited more rapid ex vivo LDL oxidation. In another study, drinking seven mL cranberry juice per kg body mass per day for two weeks induced a significant increase in plasma antioxidant capacity (AOC) (6.5%, $P < 0.02$) and reduction in circulating oxidized LDL content ($\sim -10\%$, $P < 0.02$) in male volunteers (Ruel et al., 2005). In the same research program, consumption of 250 mL per day of "lite" cranberry juice cocktail for four weeks significantly ($p < 0.01$) raised HDL levels in obese male volunteers (Ruel et al., 2006). Additionally, during consumption of a cranberry supplement (spray-dried cranberry juice in gelatin capsules, 1200 mg/day)

advanced oxidation protein products (AOPP) in plasma decreased significantly ($p < 0.05$) after four and eight weeks, with greater than four-fold reductions relative to baseline and placebos levels. Incredibly, the reduction in AOPP remained significant ($p < 0.05$, compared to baseline) in a follow-up analysis 240 days after supplementation was stopped (Valentova et al., 2007). A measure of in vivo oxidative stress, AOPP is higher in those with coronary artery disease and ischemic heart disease than in healthy individuals (Witko-Sarsat et al., 1996; Kaneda et al., 2002). This is the first and only report to date that consumption of plant antioxidants (other than vitamin C) affects this biomarker. (Valentova et al., 2007).

In direct contrast, regular consumption of cranberry juice of 750 mL/day for two weeks had no significant effect on several cardiovascular disease risk factors (plasma AOC as measured by FRAP, LDL content, and HDL content) in healthy females (Duthie et al., 2006). Ruel et al. (2006) speculate that these discrepancies may be due to the short duration of the intervention and the populations studied. Also, markers of antioxidant effects may have been missed because blood and urine were collected after overnight fasting. Increased FRAP was reported to be detectable only up to 250 minutes after cranberry juice consumption (Pedersen et al., 2000).

Overall, then, most available data suggests that regular consumption of cranberry products increases the antioxidant capacity and decreases oxidation biomarkers in human plasma and thus may contribute actively to maintaining cardiovascular health. More detailed clinical evaluations should reveal whether cranberry supplementation may become therapeutic as well as preventative in high risk populations, e.g. hypercholesterolemic, hypertensive, or obese patients.

It must be noted, however, that in none of the studies cited were plasma levels of cranberry phenols measured, and as will be discussed in Section titled Bioavailability and Metabolism of Cranberry Phytochemicals, absorption of cranberry flavonoids is extremely low. Thus, it seems most likely that cranberry consumption mediates protection of the cardiovascular system indirectly and at a distance rather than by direct molecular intervention within the blood vessels. In particular, the anti-inflammatory effects as well as signal transduction cascades that activate physiological defenses may play critical roles in cardio-protection by cranberries. These mechanisms will be discussed in further detail in Sections titled Anti-Inflammation and the Effects on Signal Transduction, Protein Expression and Activity.

Neurological Health

Until recently, the cause of age-related loss of neurological function defied convincing explanation. Then, the inability of aged brain tissue to counter oxidative insults was hypothesized as the primary cause of this degeneration (Markesbery, 1997). Applied to Alzheimer's disease, this theory holds that oxidative stress, largely facilitated by amyloid-β accumulation and subsequent free radical formation, initiates a cascade of events that mediate Ca^{2++} efflux from neurons and lead to further oxidative stress and eventually cell death. Clinical and epidemiological observations have emerged to support this reasoning (Engelhart et al., 2002; Petersen et al., 2005).

Dietary antioxidants may be effective in inhibiting this process. Indeed, animals fed polyphenol-rich berries and grapes (or extracts of these) showed improved neural function, marked by inhibition and even reversal of age-related neural deficiencies as measured behaviorally and physiologically (Joseph et al., 1999; Bickford et al., 2000; Andres-Lacueva et al., 2005; Shukitt-Hale et al., 2006a, 2006b), and this effect may extend to protection from ischemic damage and radiation-induced neural damage (Wang et al., 2005; Rabin et al., 2005). Perhaps most convincingly, a prospective study of 1836 subjects demonstrated that increased consumption of fruit and vegetable juices was inversely correlated with development of Alzheimer's disease (Dai et al., 2006). Subjects who consumed less than one serving per week of fruit and vegetable juices were four times more likely to develop Alzheimer's disease than those consuming three or more servings per week ($p < 0.01$). Intake of vitamins E and C as well as β-carotene failed to correlate with Alzheimer's disease development, so nonnutrient phytochemicals, polyphenols in particular, were proposed to be the components responsible.

Although blueberries seem to be the prototype for this effect, neuroprotection also has been associated with cranberries. In a model of Alzheimer's disease in which COS-7 cells were treated with dopamine or amyloid-β, pretreatment with a cranberry extract significantly reduced deficits of Ca^{2+} homeostasis compared to controls (Joseph et al., 2004). In addition, an anthocyanin-rich cranberry extract inhibited oxidation of the neurotransmitter 6-hydroxy-dopamine in an in vitro cellular model of Parkinson's disease (Yao and Vieira, 2007).

In an animal study of cranberry effects on Alzheimer's disease, aged rats fed 2% (by weight) freeze-dried cranberries for eight weeks showed greater strength and balance than controls in tests of motor skills ($p = 0.0001$). Brain tissue from the group fed cranberries exhibited enhanced nerve signal transmission ($p = 0.007$) and better response ($p = 0.06$) towards oxidative stress ex vivo after 16 weeks supplementation. The investigators concluded that cranberries enhanced neural function, neuro-protective responses, and some motor function in these aged animals (Shukitt-Hale et al., 2005). In humans, on the other hand, a double-blind, placebo-controlled pilot study found no significant correlation between improved neuropsychological function and cranberry juice cocktail supplementation (32 ounces, ~950 mL per day) for six weeks in fifty elderly participants, although a positive trend ($p = 0.12$) was observed in self-reported improved memory function (Crews et al., 2005). The researchers suggest that a larger trial over a more extended period may more effectively reveal the neuroprotective actions of cranberries.

Although it has received relatively little attention, protection of neurological function seems to be a promising area of research for cranberries and other foods high in antioxidants. More extensive testing in elderly human populations, with more

subjects and longer periods of supplementation, certainly seems appropriate to verify or disprove this potential benefit of cranberry consumption.

Anticancer

Cancer, one of the most feared diseases and now the #1 killer of those under 85 (Twombly, 2005), involves DNA mutation and unchecked tumor growth as major mechanisms. Current radiation and chemotherapy treatments are often ineffective against tumors and cause damage to peripheral tissues, so finding alternative therapies as well as means of prevention is of paramount importance. For the past thirty years, diet has been a major focus in cancer prevention research, and isolated phytochemicals are being evaluated as chemotherapeutic agents or aids to current chemotherapies.

Although connections between diet and cancer, either positively or negatively, were initially treated as folklore fantasy, there is now strong epidemiological evidence supporting reduction of cancer incidence and mortality by high dietary consumption of fruits and vegetables (Ames and Gold, 1991; Block et al., 1992; Ames et al., 1993; Ruxton et al., 2006; Linseisen et al., 2007). Meta-analysis of case control studies revealed strong inverse correlation between fruit consumption and esophageal, stomach, colorectal, bladder, and lung cancer risk, but not breast cancer (Riboli and Norat, 2003). Effects were most dramatic with stomach cancer incidence and least with colorectal cancer (respective odds ratios odds ratio 0.69 and 0.93 per 100 g fruit consumed per day). Results of epidemiological studies relating total flavonoid consumption to cancer rates have been mixed, hampered by incomplete knowledge of the occurrence and distribution of these phytochemicals in foods and also in tissues (Hollman and Katan, 1999; Erlund, 2004).

In vitro studies have revealed encouraging potential for cranberry products, extracts, and individual components to inhibit cancer (see Neto et al., 2007b for a recent comprehensive review). Quercetin seems to be particularly active in vitro, suppressing proliferation in pancreatic, leukemia, colon, and breast cancer cells by 50% at 15–60 μg/mL (Choi et al., 2001; Lee et al., 2002; Neto, 2007a, 2007b). Cell growth was also inhibited by 50% when exposed to ursolic acid derivatives (42–117 μg/mL) in lung, cervical, breast, colon, prostate, and leukemia cancer models (Murphy et al., 2003) and by cranberry proanthocyanidins (20–70 μg/mL) in lung, cervical, and leukemia cancer cell lines (Neto et al., 2006). Proanthocyanidin-rich extracts proved cytotoxic to ovarian cancer cells at concentrations as low as 79 mg/mL and significantly increased the chemotherapeutic activity of paraplatin (Singh et al., 2007).

Evidence for anti-cancer effects of cranberries in vivo is more equivocal. Feeding studies of cranberry components, most notably large quantities of quercetin and resveratrol, demonstrate clear inhibition of induced cancers in rats and mice (Verma et al., 1988; Baur and Sinclair, 2006). Resveratrol feeding (200 μg/kg per day for 100 days) lowered the incidence of azoxymethane-induced precancerous aberrant crypt foci in rats by ~35% ($p < 0.01$) compared to controls (Tessitore et al., 2000). Cranberry juice given as 20% solutions versus water controls, <20 mL/day, reduced the incidence of azoxymethane-induced aberrant crypt foci in rats by 77% ($p > 0.05$) when supplemented for three weeks before and ten weeks after the chemical insult (Boateng et al., 2007).

In human clinical trials, aspirin (an acetylated derivative of salicylic acid found in cranberries) at as low as 81 mg/day dose-dependently decreased precancerous, colorectal adenomas in patients with a confirmed history of adenoma incidence within 16 months prior to the study (Baron et al., 2003). Higher antioxidant capacitates in the plasma of healthy male and female volunteers consuming cranberry juice (Pedersen et al., 2000; Ruel et al., 2005; Lu and Wang, 2006) is also suggestive of increased anti-cancer potential. On the other hand, in healthy females cranberry supplementation for six weeks failed to alter biomarkers of cancer, such as plasma antioxidant capacity, levels of basal lymphocyte DNA damage in vivo, and induced lymphocyte DNA damage ex vivo (Duthie et al., 2006). However, blood samples were collected after overnight fasting. This protocol would miss effects if cranberry compounds and damaged DNA are cleared or metabolized in less than twelve hours. Thus, it would be interesting to see if cancer biomarkers are altered within short times after consumption and to determine how long any protection lasts. It would also be useful to determine whether groups with higher cancer risk (such as cigarette smokers) show greater response to cranberries.

The potential for anti-cancer action when cranberry compounds contact cancer cells seems clear, although for this protection to be actuated in vivo, the active compounds must be absorbed and distributed to tissues, a distinct problem for several major cranberry components. However, the requirement for absorption is eliminated in the gastrointestinal tract, which may explain why cranberries seem to be particularly protective against colon cancer.

Issues of uptake and bioavailability as well as possible mechanisms of action will be discussed further in Sections titled Bioavailability and Metabolism of Cranberry Phytochemicals and the Effects on Signal Transduction, Protein Expression, and Activity.

Anti-Inflammation

Inflammation, the physiological response to real or perceived harmful stimuli or infection, is critical to immune function, but when out of control it produces chronic inflammatory conditions such as arthritis, colitis, and periodontitis. In inflammation, immune cells are attracted to foreign bodies or abnormal cells and attempt to remove them. Recruitment of immune cells and regulation of inflammation is complex; the total response involves cyclooxygenase enzymes (COX-1, COX-2) that produce eicosanoids, nitric oxide, and histamine as well as

adhesion molecules (ICAM-1, VCAM-1), nuclear factors (NF-κB), chemokines (IFN-γ, TNF-α, IL-1), and cytokines (IL-6, IL-8). Faulty regulation, persistent infection, and autoimmunity lead to chronic inflammation: immune cells release reactive nitric oxide and potent enzymes that cause tissue damage and promote apoptosis, leading to cell death.

Inflammation appears to play an important exacerbating role in diseases such as cancer and atherosclerosis. For example, chronic inflammation predisposes tissue to cancer development (Hagemann et al., 2007). Chronic activation of the epithelium resulting in a variety of inflammatory responses is pivotal in recruiting immune cells to blood vessel walls in the development of atherosclerosis (Ross, 1999). Considering this behavior, some have proposed that plasma levels of adhesion molecules are novel, dynamic biomarkers of endothelial dysfunction and elevated levels of these are risk factors for atherosclerosis (Ruel and Couillard, 2007).

Investigations of diet and phytochemical effects on inflammation mediators reveal potent anti-inflammatory activity in cranberries and its components in vitro (Kandil et al., 2002; Youdim et al., 2002a; Bodet et al., 2006a, 2007a, 2007b). Anthocyanins and hydroxycinnamic acids isolated from cranberries reduce inflammation responses in human microvascular endothelial cells challenged with TNF-α, in particular limiting upregulation of cytokines and adhesion molecules (Youdim et al., 2002a). Regular cranberry juice consumption for twelve weeks in obese volunteers lowered levels of plasma adhesion molecules (Ruel and Couillard, 2007). In inhibiting immune responses from vascular endothelial cells, these results suggest cardioprotective as well as anti-inflammatory benefits from cranberry components (Youdim et al., 2002a; Ruel and Couillard, 2007). Cranberry NDM, in a dose-dependent manner, also inhibited the inflammatory responses of human macrophages induced by periodontal pathogen lipopolysaccharides (Bodet et al., 2006a) (see also the Section titled Dental Health and Gum Disease).

In vivo, several isolated cranberry phytochemicals, including flavonols, salicylic acid, resveratrol, and triterpenoids inhibit inflammation in animal models. Quercitrin, one of cranberry's more abundant flavonols, reduced an index based on physiological observations (body weight, presence of blood in feces, and stool consistency) and ex vivo cytokine production in a rat model of colitis (Comalada et al., 2005). Also in rats, resveratrol inhibited inflammatory COX activity both before and after induction by N-nitrosodiethylamine (Khanduja et al., 2004). One patent application claims that flavonols and purified cranberry phytochemicals reduce inflammation in vivo. Mouse ear edema induced by 12-O-tetradecanoylphorbol-13-acetate was reduced by 34.0% and 55.1% after topical application of 87.5 μg and 175 μg of quercetin-3-O-(6″-benzoyl)-β-galactoside isolated from processed cranberry powder (Vorsa et al., 2007b).

Overall, the potential for dietary consumption of cranberry products to modulate inflammation appears promising, although more in vivo studies are needed for confirmation and for identification of necessary levels and active compounds. Possible mechanisms involved in anti-inflammatory effects of cranberries will be discussed further in the Section on Effects of Signal Transduction, Protein Expression, and Activity.

BIOAVAILABILITY AND METABOLISM OF CRANBERRY PHYTOCHEMICALS

Bioavailability is currently a hot topic in nutrition and food research, and this increased interest is well-justified: for a given component to affect an organ, it must reach that tissue in active form at biologically relevant concentrations. The in vitro studies commonly used to screen antioxidant activity of phytochemicals present in foods and natural products may reveal inherent reactivity. However, the results are useless for predicting or explaining bioactivity if the compounds of interest are poorly absorbed, metabolized into different products after consumption, or otherwise never reach the target organs intact or at relevant concentrations.

Relatively little is yet known concerning the absorption efficiency and bioavailability of cranberry phytochemicals after consumption by humans or animals, although new data is beginning to emerge. The most definitive information comes from studies of isolated phytochemicals in animal models. Table 5 summarizes the bioavailability of some classes of polyphenols (found in cranberry) as isolated compounds and from various foods. These observations offer broad insights but may be misleading if used to draw specific conclusions regarding the dietary intake of cranberries. The food matrix and interactions between food components strongly influence bioavailability (Parada and Aguilera, 2007). For example, observations of flavonol bioavailability vary wildly depending on many factors including the source, the specific molecular species, the presence of fats, and the degree of processing of the food consumed (Hollman and Katan, 1999; Aherne and O'Brien, 2002; Parada and Aguilera, 2007). Known plasma metabolites of cranberries (as well as intact phytochemicals shown to reach the bloodstream) are listed in Table 6. The few compounds found in urine after cranberry consumption are detailed in Table 7.

Anthocyanin bioavailability is generally poor compared to other flavanoids (Manach et al., 2005), usually limited to less than a percent of the amount ingested. However, about 5% of ingested anthocyanins were recovered intact in the urine of volunteers fed cranberry juice, mostly within 3–6 hours after ingestion (Ohnishi et al., 2006). Substantially higher than the $\sim$1% urinary excretion of anthocyanins from wine and strawberries (Manach et al., 2005) and the highest reported for anthocyanins from any food source, this unusual absorption was attributed to interactions of anthocyanins with other components present in the juice, promoting increased absorption (Ohnishi et al., 2006). Peonidins accounted for greater percentages of recovered anthocyanins than did cyanidins, which suggests partial metabolism (*O*-methylation) of the cyanidin-glycosides (Ohnishi et al., 2006). No nonanthocyanin metabolites were identified. In contrast, two recent studies suggest minimal or no urinary excretion

Table 5 Bioavailability of phenolic classes (pure form or from various foods) found in cranberries

Phytochemical Class	Peak Plasma Conc.	T_{max} (h)	Tissues Where Available	Primary Forms Detected In Vivo	Urinary Excretion*	References
Anthocyanins	0.001–0.2 μM	$\sim$ 0.7–4	Brain	intact, methylated	0.004—5.1%	Milbury et al., 2002; Andres-Lacueva et al., 2005; Manach et al., 2005; Ohnishi et al., 2006
Flavonols	0.05–7.6 μM	0.5–9.3	lungs, testes, liver, kidney, heart	intact, glucuronided, methylated, sulfated	0.07–7%	Erlund, 2004; de Boer et al., 2005; Manach et al.,2005, Vorsa et al., 2007
Catechins	0.03–5.9 μM	0.4–4	liver, kidney	intact, methylated	0.1–55%	Manach et al., 2005; Tsang et al., 2005
PAC dimers	ND-41 nM	2	—	intact	ND-1.0%	Prior and Gu, 2005; Tsang et al., 2005
PAC trimers and polymers	ND	ND	ND	phenolic acids, monomers (metabolism in intestine)	ND	Prior and Gu, 2005; Tsang et al., 2005; Valentova et al., 2007
Phenolic Acids	ND-40 μM	0.5–3	—	intact, methylated glycine conjugated	27%–39.6%	Scalbert and Williamson, 2000; Zhang and Zuo, 2004; Manach et al., 2005
Trans-Cinnamic Acids	0.006–40 μM	0.5–3	—	intact, glucuronided, methylated, sulfated	0.3–61.7%	Bourne and Rice-Evans, 1998; Rechner et al,. 2001a; Rechner et al., 2001b; Manach et al., 2005
Stilbenes (resveratrol)	ND-32 μM	4	liver, kidney	glucuronided, sulfated	2.3%	Meng et al., 2004; Baur and Sinclair, 2006

of anthocyanins after regular consumption of cranberry products (Duthie et al., 2006; Valentova et al., 2007).

Differences in sample handling may contribute to discrepancies in anthocyanin detection. Collection of urine directly into an acidified solution (Ohnishi et al., 2006) prevents degradation of anthocyanins, whose stability is highly pH-dependent. Given evidence that cranberry anthocyanins are excreted quickly, urine collection after overnight fasting (Duthie et al., 2006) is likely to miss most or all of the compounds absorbed. Freeze-drying urine prior to analysis (Valentova et al., 2007) may also lead to degradation of anthocyanins, especially if samples are not protected from light.

Anthocyanins are unique in that they are the only flavanoid absorbed primarily in the stomach, mostly in glycosylated forms (Mazza et al., 2002; Talavera et al., 2003). Anthocyanins also have been shown to pass the blood brain barrier. Cyanidin-3-galactoside, cyanidin-3-arabinoside, and peonidin-3-arabinoside known to be prevalent in cranberries were identified in rat brains after consumption of blueberries (Andres-Lacueva et al., 2005). The same anthocyanins should

Table 6 Cranberry phytochemicals and metabolites found in plasma after cranberry consumption

Molecular Species	Source	Duration	Dose of Compound	Cmax	Tmax (m)	References
Benzoic Acid*	CJC, 200 mL	single exp.	11 mg	36	45	Zhang and Zuo, 2004
Salicylic acid*	CJC, 200 mL	single exp.	620 μg	7.1	45	Zhang and Zuo, 2004
Total Salicylates*	CJC, 750 mL/day	2 weeks	5.28 mg/day	0.23**	ND***	Duthie et al., 2005
2,3-Dihydroxybenzoic acid*	CJC, 200 mL	single exp.	482 μg	13	45	Zhang and Zuo, 2004
2,4-Dihydroxybenzoic acid*	CJC, 200 mL	single exp.	ND	5.5	270	Zhang and Zuo, 2004
p-Hydroxyphenylacetic acid*	CJC, 200 mL	single exp.	ND	8.2	45	Zhang and Zuo, 2004
Ferulic acid*	CJC, 200 mL	single exp.	220 μg	1.6	270	Zhang and Zuo, 2004
Sinapic acid*	CJC, 200 mL	single exp.	1.0 mg	6.7	270	Zhang and Zuo, 2004
Ascorbic Acid	CJC, 500 mL	single exp.	134 mg	0.2**	240	Pedersen et al., 2000
Ascorbic Acid	CJC, 750 mL/day	2 weeks	675 mg/day	60**	ND***	Duthie et al., 2006
Total Phenolics	CJC, 500 mL	single exp.	450 mg	3527	60	Pedersen et al., 2000
Quercetin-3-galactoside	Isolated compound	single exp.	NA	NA	7.5	Vorsa et al., 2007a
Quercetin-3-glucoside	Isolated compound	single exp.	NA	NA	15	Vorsa et al., 2007a

Abbreviations: Cmax = Maximum plasma concentration; Tmax = Time to maximum concentration; exp. = exposure; m = minutes; ND = not determined; NA = information not provided in reference; *denotes samples hydroyzed prior to analysis; **denotes increase from basal levels; ***plasma collected after overnight fasting.

Table 7 Compounds found in urine after cranberry consumption

Molecular Species	Source	Duration	Dose of compound	Amount Observed in Urine	% of Dose	References
Cyanidin-3-*O*-galactoside	CJ (200 mL)	Single Exp.		4.70 μg/24 h	3.7	Ohnishi et al., 2006
Cyanidin-3-*O*-glucoside	CJ (200 mL)	Single Exp.	3.6 μg	0.0492 μg/24 h	1.4	Ohnishi et al., 2006
Cyanidin-3-*O*-arabinoside	CJ (200 mL)	Single Exp.	104.6 μg	3.64 μg/24 h	3.6	Ohnishi et al., 2006
Peonidin-3-*O*-galactoside	CJ (200 mL)	Single Exp.	174.3 μg	19.1 μg/24 h	11.0	Ohnishi et al., 2006
Peonidin-3-*O*-glucoside	CJ (200 mL)	Single Exp.	9.9 μg	1.11 μg/24 h	11.3	Ohnishi et al., 2006
Peonidin-3-*O*-arabinoside	CJ (200 mL)	Single Exp.	230 μg	4.50 μg/24 h	2.0	Ohnishi et al., 2006
Total Anthocyanins	DCJ (1200 mg/day)	8 wk.	NA	<0.7 mg/mmol creatinine*	NA	Valentova et al., 2007
Quercetin-3-galactoside	Isolated compound	Single Exp.	NA	NA	NA	Vorsa et al., 2007a
Myricetin-3-galactoside	Isolated compound	Single Exp.	NA	NA	NA	Vorsa et al., 2007a
Quercetin glucuronide	DCJ (1200 mg/day)	8 wk.	NA	$\sim$ 1 mg/mmol creatinine*	NA	Valentova et al., 2007
Hippuric acid	DCJ (1200 mg/day)	8 wk.	NA	$\sim$ 6 mg/mmol creatinine*	NA	Valentova et al., 2007
Salicyluric acid and isomers	DCJ (1200 mg/day)	8 wk.	NA	$\sim$ 3 mg/mmol creatinine*	NA	Valentova et al., 2007
Dihydroxybenzoic acids and isomers	DCJ (1200 mg/day)	8 wk.	NA	$\sim$ 0.5 mg/mmol creatinine*	NA	Valentova et al., 2007
Salicyluric acid and isomers	CJC (750 mL)	2 wk.	NA	$\sim$ 1.4 μg/mg creatinine*	NA	Duthie et al., 2005
Total Salicylates	CJC (750 mL)	2 wk.	5.28 mg/day	$\sim$ 0.21 μg/mg creatinine*	NA	Duthie et al., 2005

Abbreviations used: CJ = cranberry juice; DCJ = dried cranberry juice; CJC = cranberry juice cocktail; Exp = exposure; wk = week; NA = not available. All studies were in humans except for Vorsa et al. (2007a), which was in mice.

*Results from urine analyses on final day of treatment; urinary concentrations expressed relative to creatinine to account for variation in urinary volume; reported as difference between treatment and placebo levels. These could not be converted to absolute concentrations because urinary volumes were not available.

be available to the brain upon consumption of cranberries, albeit in very small concentrations.

Further investigation is needed to confirm the report of high anthocyanin availability and urinary excretion from cranberry juice, and to identify anthocyanin metabolites and tissue distributions, if any.

Proanthocyanidins exhibit poor bioavailability. Some speculate that metabolites of PACs may be absorbed and elicit health effects in the urinary tract (Howell, 2007). Reports of PAC bioavailability focus on grape seed, cocoa products, and purified isolates while cranberry PAC bioavailability data is mostly lacking, perhaps because they are not absorbed. Some in vitro investigations suggest that smaller PAC oligomers and polymers are absorbed in small quantities through models of the intestinal epithelium, but this uptake is limited mostly to cleaved monomer units (Deprez et al., 2001; Spencer et al., 2001a, 2001b). Other data suggests that gut microflora significantly degrade polymeric PACs to aromatic acids, which may be absorbed in more significant quantities (Deprez et al., 2000). One such microbial metabolite, *p*-hydroxyphenylacetic acid, was found in human plasma 90 and 270 minutes after a single administration of cranberry juice, while it was not present in the juice (Zhang and Zuo, 2004). This metabolite, however, may also arise from gut degradation of any of cranberries flavonoids.

In vivo data regarding the fate of PACs after absorption present a mixed picture. Intact PACs are not absorbed at all in most animal models (Donovan et al., 2002). Nearly 100% of radioactivity was recovered in feces of chicken and sheep after being fed PAC oligomers and polymers, showing virtually no uptake or metabolism of any kind (Jimenez-Ramsey et al., 1994; Terrill et al., 1994). One investigation found no PACs in human urine after eight weeks of supplementation with dried cranberry juice (Valentova et al., 2007). Trace amounts of PAC dimers and trimers were found in urine of rats after consumption of large quantities of PAC-rich grape seed extract (Koga et al., 1999; Tsang et al., 2005). PACs from grapes, however, contain only B-type linkages, so are not direct models of the A-type PACs common in cranberries.

PACs are known to associate with diverse biological elements, including proteins and LDL cholesterol (Porter et al., 2001). Along with their structural heterogeneity and the lack of commercially available standards, this complexation complicates their analysis in biological matrices and may contribute to underreporting of PAC absorption. The tannic nature of PACs suggests that, if absorbed, these phytochemicals would rapidly interact with and denature proteins, which would impair physiological function and potentially cause toxicity. This is a logical reason for natural design that prevents PAC absorption. However, these phytochemicals remain available within the stomach, colon, and other sections of the digestive tract, where they exhibit potent antipathogenic, antioxidant, and other biological activity, as will be discussed more in Section titled Molecular Mechanisms Underlying Health Effects.

Flavan-3-ol bioavailability after cranberry consumption has not been studied systematically. In one report, flavan-3-ol monomers were not detected in the plasma of volunteers 45 or 90 minutes after ingestion of 1800 mL of cranberry juice cocktail (Zhang and Zuo, 2004). However, with such late sampling times any absorbed monomers may well have been missed due to rapid metabolism and elimination or distribution. Flavan-3-ol monomers from other fruits are absorbed similarly to flavonols, then metabolized to sulfate or glucuronic acid conjugates as reviewed by Manach et al. (2005).

Generally, flavonols seem to be more bioavailable than other cranberry flavonoids. Studies with purified flavonol isolates in animal models generally show that $\sim$10% of consumed flavonols are absorbed, and that absorption and metabolism are highly dependent on flavonol structure, presence and type of

glycosidic linkage, and the food matrix (Erlund, 2004; Manach et al., 2005; Parada and Aguilera, 2007). Feeding large quantities of quercetin to rats (0.1% and 1% diet by weight) and pigs (500 mg/kg) resulted in greatly enhanced absorption, extensive metabolism, and distribution to a variety of tissues (de Boer et al., 2005). With the 0.1% dose, quercetin was found in plasma (7.70 μM), lungs (1.04 μmol/kg), testes (0.82 μmol/kg), kidneys (0.93 μmol/kg), and heart (0.50 μmol/kg) with lesser amounts in other tissues. While only unmetabolized quercetin was detected in plasma, metabolites predominated in tissues. Absorbed cranberry flavonols are excreted in bile and urine, largely as glucuronidated metabolites (Valentova et al., 2007; Vorsa et al., 2007a). Indeed, flavonols accumulate, intact and as glucuronidated metabolites, in the urethra of mice (Vorsa et al., 2007a). Cranberry flavanols, among the most abundant and most bioavailable flavonoids in cranberry products, have been surprisingly overlooked in attributions of health effects.

Smaller, simple phenolics appear to be among the most well absorbed of cranberries nonnutrient phytochemicals. Indeed, cranberry phenolic acids, relatively small and simple molecules, have even greater bioavailability than flavanols. Total phenolics increased significantly to ~600 mg/L gallic acid equivalents above basal levels in human plasma following the consumption of 500 mL of cranberry juice (Pedersen et al., 2000), mostly due to phenolic acids and hydroxycinnamic acids. μg/mL concentrations of benzoic acid, 2,3-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, *o*-hydroxybenzoic acid, ferulic acid, and sinapic acid were detected in human plasma after cranberry juice consumption (Zhang and Zuo, 2004). Phenolic acids identified in human urine in significantly higher concentrations than controls after administration of dried cranberry juice include hippuric acid, salicyluric acid and its isomers, and dihydroxybenzoic acid and its isomers (Valentova et al., 2007). Levels of salicylates in the urine and plasma of volunteers tripled from basal levels following two weeks of daily cranberry juice consumption (Duthie et al., 2005). To not overread these reports, however, it must be stressed that absorbed levels in all cases were very low, as shown in Table 5.

Resveratrol, in the only available investigation of its bioavailability from cranberry consumption, was not detected in the plasma of volunteers 45 minutes after a single administration of cranberry juice (Zhang and Zuo, 2004). Other investigations of resveratrol metabolism, however, suggest it is mostly converted to sulphonated and glucuronated derivates within 30 minutes (Walle et al., 2004), so sampling after longer times is too late. Studies monitoring metabolized forms of this bioactive polyphenol within minutes after cranberry consumption may be more sensitive, especially since quercetin has been shown to drastically slow its metabolism (Baur and Sinclair, 2006).

There is no question about the bioavailability of ascorbic acid in cranberries. Indeed, markedly increased levels of plasma vitamin C have been observed after the consumption of cranberry products. A single administration of 500 mL of cranberry juice cocktail significantly elevated plasma vitamin C to ~20 μM within 2 hours, and levels were maintained for 250 minutes post consumption. After two weeks consumption of 750 mL cranberry juice per day by healthy volunteers, vitamin C levels remained significantly increased (~30 μM) even after overnight fasting (Duthie et al., 2006).

Given their in vitro bioactivities, the fates of ursolic acid and other polyphenolics after cranberry consumption warrant additional research; likewise, continued research into the bioavailabilities of the flavonoid fractions of this fruit seems justified, particularly to clarify their influence on UTIs, plasma antioxidant capacity, inflammation, and cancer. The bioavailability of other cranberry components is either irrelevant or unknown. Essential vitamins and minerals found in cranberry products are undoubtedly absorbed and their well-documented health effects are beyond the scope of this paper. Sugars and nonphenolic organic acids, too, are well absorbed but do not exhibit important health effects. Complex carbohydrate fibers in cranberries are, in all likelihood, not absorbed.

MOLECULAR MECHANISMS UNDERLYING HEALTH EFFECTS

As a complex mixture of phytochemicals, cranberry products induce their observed biological effects via a multitude of molecular mechanisms, sometimes competing and sometimes additive or synergistic. Mechanisms may be divided into the following three categories:

- Pathogen interactions
- Antioxidant mechanisms
- Effects on signal transduction, protein expression and activity.

Pathogen Interactions

Uncommon and perhaps unique among plant foods, cranberry exhibits clear inhibition of a variety of pathogens, both in vitro and in vivo, with implications for *E. coli* in the bladder, *H. pylori* in the stomach, numerous oral bacteria, and viruses. The components and mechanisms responsible for the anti-pathogenic effects of cranberries remain controversial, but recent observations provide some interesting insights.

Acidification of urine and increase in urinary volume were early mechanisms proposed to explain how cranberries prevent UTIs, but these have been disproven. Placebo controlled studies preclude the latter mechanism and observations of no significant change in urinary pH after consumption of cranberry products dispel the former (Avorn et al., 1994; Walker et al., 1997; Di Martino et al., 2006; Valentova et al., 2007).

Interference with bacterial adhesion is now believed to account for much of anti-pathogenic actions of cranberries (Sobota, 1984; Howell, 2007). In vitro investigations suggest that cranberry phytochemicals interact with bacterial cell surface proteins, including those responsible for adhesion, biofilm formation and acid tolerance (Zafriri et al., 1989; Ofek et al., 1991;

Howell et al., 1998; Foo et al., 2000a; Steinberg et al., 2005; Duarte et al., 2006; Liu et al., 2006). These in vitro observations point to NDM, PACs, flavonols, and fructose as the active components, as indeed they probably are in the mouth, stomach, and the digestive tract. However, negligible absorption of NDM and PACs, as well as placebo-controlled studies for fructose precludes their activity in the urinary tract.

The interference of cranberries with bacterial adhesion has been observed in a variety of cells and biological matrices, including uroepithelial cells (Sobota, 1984), vaginal epithelial cells (Gupta et al., 2007), gastric cells (Shmuely et al., 2004), erythrocytes (Zafriri et al., 1989), buccal cells (Sharon and Ofek, 2002), gastric mucus (Burger et al., 2000) and tooth-like surfaces (Weiss et al., 2004), as well as in nonbiological matrices such as silicone rubber (Habash et al., 1999), glass (Allison et al., 2000), and borosilicate coated glass (Johnson-White et al., 2006). Many bacteria are affected including a large number of gram-negative oral bacteria (Weiss et al., 1998, 2002), *S. mutans* (Duarte et al., 2006), *P. gingivalis* (Bodet et al., 2006b), *E. coli* (Sobota, 1984), and *H. pylori* (Burger et al., 2000), but not *Campylobacter jejuni* (Johnson-White et al., 2006), *Lysteria monocytogenes* (Johnson-White et al., 2006), or diarrheal isolates of *E. coli* (Ofek et al., 1991). That cranberries are effective in so many different systems with different structures and molecular components yet act against a relatively limited set of bacteria strongly argues that the anti-adherence activity of cranberry stems from interaction with the microorganism rather than interaction with the matrix.

Indeed, the anti-adherence effects of cranberries appear to be lectin specific (Sharon and Ofek, 2002). But how do cranberry phytochemicals block adherence? Six discrete mechanisms that have been proposed merit discussion:

- promotion of high urinary concentrations of the adherence-inhibiting Tamm-Horsfall glycoprotein (Zafriri et al., 1989)
- alteration of electrostatic properties (Habash et al., 2000)
- change in shape of pathogenic bacteria (Ahuja et al., 1998)
- action as receptor analogs (Zafriri et al., 1989; Howell, 2007)
- reduction of bacterial lectin expression (Ahuja et al., 1998)
- denaturation of bacterial lectins (Liu et al., 2006)

Endogenous Tamm-Horsfall glycoprotein, the most prevalent protein in normal human urine, has been linked to innate defense against urinary pathogens (Tamm and Horfall, 1950). This protein inhibits adherence of *E. coli* to kidney cells (Dulawa et al., 1988; Kumar and Muchmore, 1990). Moreover, Tamm-Horsfall protein knockout mice had UTIs longer in duration with a higher degree of colonization after inoculation with type-1 *E. coli* than control mice capable of expressing this protein (Bates et al., 2004). Following this pattern, increased T-H protein could explain why, after cranberry consumption, urine inhibits adherence of type-1 *E. coli* ex vivo. That cranberry phytochemicals may promote Tamm-Horsfall glycoprotein expression is appealing in its mechanistic simplicity, but unfortunately there is no direct evidence yet to substantiate this action. In particular, increases in Tamm-Horsfall protein in urine after cranberry consumption have not been observed. Furthermore, in vitro investigations suggest cranberry interaction with the pathogen rather than with the host. Still, this mechanism has not been investigated systematically so further research is warranted before it is dismissed.

The alteration of zeta potential of uropathogenic bacteria has been observed in only one study, but deserves consideration as a mechanism of cranberry anti-pathogenic effects. Hydrophobic interactions are thought to be critical forces in bacterial lectin adhesion to host receptors (Magnusson, 1982). Charges on a cell surface (the zeta potential) interfere with hydrophobic associations and add electrostatic interactions, and thus may be an important factor counterbalancing the pathogenic bacteria's ability to bind to host receptors. For example, positive changes to the zeta potential of several uropathogens, including *E. coli*, observed after incubation in urine of volunteers who consumed cranberry supplements for three days was assumed to prevent pathogen binding by creating an electrostatic repulsion on cells (Habash et al., 2000), although the components and mechanisms responsible for this change were not identified. Zeta-potential has never been linked directly to microbial adherence, but the phenomenon merits further investigation and consideration in detailed focused studies.

Mechanistic studies of the effects of cranberries on lectins have documented considerable changes in the shape of uropathogens, which logically may be expected to alter docking fit at cell surfaces. In one study, *E. coli* were cultured in growth media containing 0 or 25% cranberry juice, adjusted to pH 7. Two strains of P-fimbriated *E. coli* were observed via electron microscopy as ovoid when grown in the control agar medium but as elongated rods when in grown in the presence of cranberry juice (Ahuja et al., 1998). It would be interesting to see if such morphological changes are also observed when *E. coli* are incubated in urine from subjects consuming cranberry juice. How shape changes might affect adhesion is not yet known and has not been evaluated (Howell, 2007), but it may be indicative of stress to the bacteria. Morphologic alterations are not likely the main mechanism underlying the anti-adherence activity of cranberries, but may contribute to creation of an unfriendly environment that is not conducive to microbial "settling in."

Another possible mechanism is that phytochemicals act as receptor analogs and bind preferentially to the lectins, effectively competing with actual receptor cites (Zafriri et al., 1989; Howell, 2007). This is the most likely mechanism for adherence inhibition in vitro by fructose in cranberries (Zafriri et al., 1989), although there is no evidence that fructose consumption inhibits UTIs in vivo (Howell, 2007). In fact, other juices and foods rich in fructose and/or B-type PACs, including apple juice, grape juice, green tea, and dark chocolate, do not induce ex vivo anti-adherence in urine after consumption (Howell et al., 2005). Likewise, there is no evidence that cranberry NDM or proanthocyanidins act as surrogate receptors as they inhibit P-type *E. coli* in vitro (Zafriri et al., 1989; Howell et al., 1998). Competitive receptors, though, may be responsible for some of the observed activity on *H. pylori* in the stomach or, as suggested by Zafriri

et al. (1989), for activity against type-1 *E. coli* in the digestive tract. Indeed, a major part of UTI inhibition by cranberries may occur in the gut, the primary source of uropathogenic *E. coli* causing these infections (Zafriri et al., 1989; Kontiokari et al., 2003) rather than in the kidney or bladder reached by only trace amounts of absorbed and conjugated metabolites. Binding strains of *E. coli* with uropathogenic potential and reducing their ability to colonize in the gut may promote dominance of nonvirulent strains (Howell, 2007), which further impairs the ability of uropathogenic *E. coli* to grow and thrive. Overall, decreased intestinal levels of pathogens materially contribute to the lower incidence of UTIs and bacteriuria following cranberry consumption.

Reduction of bacterial lectin expression by cranberry phytochemicals or their metabolites as suggested by Ahuja et al. (1999) is certainly possible. For one strain of P-fimbriated *E. coli* (JR1), serial plating in a medium containing 25% cranberry juice progressively reduced the percentages of bacteria with lectins visible by electron microscopy. Agglutination also progressively diminished, suggesting depressed rates of lectin expression and adhesion. In another strain (DS17), no fimbriae were observed after plating in media containing cranberry juice, while 40% of the bacteria grown in control media without cranberries had fimbriae. From these results, it was proposed that cranberry components reduce lectin expression via several possible mechanisms, including fimbrial synthesis, fimbrial attachment to the cell wall, or phase variation (Ahuja et al., 1998). Observations that in vitro, high molecular weight constituents of cranberry juice retarded formation of extracellular glucosyl- and fructosyl- transferases, enzymes from *Streptococcus sobrinus* that facilitate dental plaque formation and adhesion of dental pathogens to teeth (Steinberg et al., 2004), supports the first two of these mechanisms. The third mechanism, phase variation, is a phenomenon observed in numerous pathogens including uropathogenic *E. coli*, wherein the bacteria express their various virulence factors such as lectins (or do not express them) at different times according to the progression of the infection and stress state (Rhen et al., 1983; Pere et al., 1987). Some component of cranberry or its metabolites, may elicit a stress response in *E. coli*, directly leading to reduced expression of lectins in the urinary tract.

Increasing evidence suggests that pathogenic bacteria communicate via quorum sensing, with dramatic influence on expression of genes that control virulence factors, including those responsible for adherence and biofilm formation (Fux et al., 2005). Via quorum sensing, pathogenic bacteria coordinate phase variation and infection based on stress levels and appropriate bacterial concentration, switching back and forth from dormant to infectious modes (Fux et al., 2005). Cranberry components or metabolites may interfere with quorum sensing compounds or proteins responsible for their regulation, ultimately leading to lowered fimbrial expression. This possibility seems very appealing, though since knowledge of quorum sensing is still rudimentary, the action of cranberries by this route has not been investigated.

The most plausible mechanism is that tannic elements of cranberry or tannic metabolites induce conformational changes in fimbriae. Atomic force microscopy has verified that within three hours exposure to cranberry juice in vitro causes significant shortening of the P-fimbriae of *E. coli* to approximately 1/3 of its original length, lowered its adhesion strength to the silicone-nitride probe, and increased its polymer density (Liu et al., 2006). While specific components responsible were not identified, the investigators concluded that binding of cranberry phytochemicals to the fimbriae followed by conformational alteration of the lectins was consistent with the observed changes. In addition, decrease in length and increase in polymer density strongly suggest protein denaturation and condensing of lectin tertiary structure, while decreased adhesion strength denotes loss of functionality. This strongly supports contentions that conformational changes to lectins, either by covalent binding or noncovalent interaction, drive the anti-adherent effects of cranberries, especially for those in the mouth, stomach, and the digestive tract. Similar examination is needed after incubation of *E. coli* in urine samples from subjects consuming cranberry to determine whether this activity relates to UTIs, but it seems possible and even probable.

If induced conformational change to lectins is the probable mechanism underlying the anti-adherence activity of cranberries, questions remain regarding responsible components. Tannins characteristically bind and often denature proteins. For a compound to be characterized as a tannin and have this functionality, it must be polyphenolic, ~500 to 3000> Daltons (Da) in size, with 1–2 hydroxyl groups per 100 Da (Chung et al., 1998). In effect, polyphenols must have enough alcohol groups to interact with hydrophilic elements of proteins as well as sufficient mass and length to interact over enough area of protein to force conformational changes. Flavonoid monomers (~300 Da), even when glycosylated (~450 Da), seem to lack the mass needed to exert tannic activity. Dimers (~600 Da, ~8 hydroxyl groups) and higher oligomers and polymers of flavonoids (including proanthocyanidins) do have appropriate mass and hydroxyl groups to mediate binding and induce protein denaturation. The isolated A-type dimers and trimers (as well as cranberry NDM and crude cranberry PAC extracts) all inhibited adherence of P-fimbriated *E. coli* (Zafriri et al., 1989; Howell et al., 1998; Foo et al., 2000a; Foo et al., 2000b). However, the B-type dimer did not have this activity in vitro nor did the urine of those having consumed other foods rich in B-type PACs ex vivo (Foo et al., 2000b; Howell et al., 2005). It has been speculated that A-type PACs have a higher degree of structural rigidity due to the extra linkage and this may play a role in the formation of unknown, bioactive urinary metabolites and/or increased binding of lectins (Foo et al., 2000a; Howell et al., 2005).

Meeting the criteria to be defined as tannins, the polymeric pigments in cranberries (or at least their proposed molecular formula) may exhibit tannic activity as well and consequently contribute to anti-adherence action. As previously mentioned, though, none of these tannic cranberry phytochemicals have been found in urine after cranberry consumption. Interestingly,

the relatively large accumulation of cranberry flavonols and their metabolites found in the urethra of mice were recently proposed to account for cranberry inhibition of UTI (Vorsa et al., 2007a). Glucuronate metabolites of flavonol glycosides do have sufficient molecular mass and enough alcohol groups to be categorized as tannins (~650 Da, ~10 hydroxyl groups), so they may be responsible for, or at least contribute to, cranberry anti- UTI action. However, isolation of the metabolites and examination of anti-adherence abilities at biologically relevant concentrations would be needed to confirm this.

Recent research of antiviral properties of cranberry phytochemcials points to cell surface interactions as another mechanism of action. One investigation suggests that cranberry phytochemicals may alter host cell surface proteins (namely junction adhesion molecule A) used by viruses as receptor sites, causing partial or total loss of infectivity (Lipson et al., 2007a). Unlike the proposed mechanisms for bacterial anti-adherence, this mechanism relies on phytochemical interaction with host cell surfaces and thus may not be viable in vivo because the key phytochemicals are poorly absorbed or not absorbed at all.

Antioxidant Activities and Mechanisms

Antioxidant mechanisms have attracted much research for their roles in preventing and potential for treating diseases that involve oxidative degradation of tissue. Decreased rates of cancer and cardiovascular disease associated with fruit and vegetable consumption have piqued attention of the general public to such an extent that produce, herbs, spices, and formulated foods are advertized for their ORAC values, the antioxidant power arising from their phytochemical components. Free radicals and reactive oxygen species produced during routine bioenergetic cellular processes, as well as electrophilic chemicals from other sources such as cigarette smoke, diet, and environmental pollutants, cause damage to vital sub-cellular components (Ames and Gold, 1991; Ames et al., 1993; Ames, 1998). Reactive chemical insults to DNA may result in mutation, apoptosis, or cancer, while damage to proteins and other structures leads to Alzheimer's disease, Parkinson's disease, cataracts, and dozens more degenerative diseases (Ames and Gold, 1991; Ames et al., 1993; Markesbery, 1997; Ames, 1998; Engelhart et al., 2002; Youdim et al., 2002b). Oxidation of LDL is an active process in the progression of atherosclerosis, and oxidative damage to specialized cells and to tissues contributes markedly to aging (Ames et al., 1993; Bickford et al., 2000).

Data presented throughout this review provide substantial evidence that consumption of cranberries leads to increased plasma antioxidant capacity and decreased biomarkers of oxidative stress. More unclear is whether phytochemicals directly quench reactive oxygen species and free radicals in vivo or whether they induce endogenous antioxidant responses through some alternate mechanism(s). In the discussion that follows, "direct" antioxidant mechanisms will be considered first, then "indirect" antioxidant mechanisms.

Direct Antioxidant Activity

The in vitro antioxidant capacity of cranberry components is among the highest reported for fruits commonly consumed in the United States (Sun et al., 2002; Wu et al., 2004). As measured by the total oxyradical scavenging assay (TOSC), cranberries have nearly twice the radical scavenging activity of the next most active fruit (Sun et al., 2002); this was attributed entirely to the phenolic content of cranberries since no vitamin C was detected (Sun et al., 2002). In a study of fourteen fruits, cranberries also had the highest total antioxidant activity by weight and second most by serving size (second to blueberry) as measured by combined hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) assays (Wu et al., 2004). Significant correlation between ORAC and ACY contents in various cranberry samples ($r = 0.869–0.929$) suggests these active pigments may also be the most powerful antioxidants in cranberries. At the same time, as might be expected, there was greater correlation between ORAC and total phenolics ($r = 0.902–0.946$) (Wang and Stretch, 2001). Indeed, abundant phenolics make cranberries one of the most antioxidant rich foods available.

These in vitro measures of antioxidant capacity are impressive but do not reveal the full story because they do not account for absorption, metabolism, distribution, and excretion of cranberry components in cells and tissues (Duthie et al., 2006). Indeed, a major limitation of cell culture and other in vitro assays is that they test direct exposures that may or may not occur in vivo. Anthocyanins and PACs, in particular, show exceptional antioxidant activity in vitro, but are poorly absorbed, as discussed earlier. Cranberry flavonoid concentrations detected in plasma, internal tissues, or organs in vivo are only nanomolar at the highest (Zhang and Zuo, 2004; Vorsa et al., 2007a). Thus, it seems unlikely that cranberry flavonoids are absorbed intact in concentrations necessary to act as direct antioxidants. The exceptions, perhaps, are the excretory organs such as the liver, kidneys, and bladder. The fast clearance observed for the flavonols and anthocyanins that are absorbed from cranberry consumption (Ohnishi et al., 2006; Vorsa et al., 2007a) may concentrate these compounds in excretory tissues for short periods. This short-term concentration, together with absorption of smaller phenols in cranberries, may cause a localized spike in redox potential following cranberry consumption, recharging endogenous antioxidants and halting damaging free radical chain reactions. The bladder, in particular, seems to be most exposed to flavonoid antioxidants as they accumulate there (Ohnishi et al., 2006; Vorsa et al., 2007a).

Poor absorption of many antioxidant phytochemicals requires a rethinking of how these compounds influence animal systems after consumption. Lack of absorption does not equate to lack of bioactivity or inability to act in vivo as an antioxidant or by other mechanisms. Cranberry phytochemicals are certainly available to the digestive tract where they may mediate a variety of responses, especially in relation to cancers and inflammatory diseases of the esophagus, stomach, and colon. Cancers of the digestive and excretory organs (esophageal, stomach, colorectal,

and bladder cancer) where cranberry phytochemicals have most contact after dietary ingestion and thus greatest opportunity for interaction are among the ones whose incidence is most inversely correlated with fruit intake (Riboli and Norat, 2003). It is likely that direct antioxidant mechanisms play a large, if not primary, role in the apparent prevention of these cancers by fruits and cranberries.

Cranberry antioxidant effects extend far beyond cancer prevention. Following cranberry consumption, the antioxidant potential of its phytochemicals reaches the intestinal tract promoting a shift to a reduced state. Modulating intestinal redox tone and associated cell signaling may be one mechanism by which cranberry antioxidants can influence physiological responses without being absorbed. Antioxidant action in the gut to counter oxidative stress there may spare endogenous antioxidants, releasing them to protect more sensitive cells and tissues against oxidative insult. Cranberry phytochemicals neutralize oxidative species in the digestive tract and by binding to molecules or to receptors on brush border epithelial may block absorption of harmful, reactive compounds.

While parent flavonoids may be poorly absorbed, there still may be an in vivo antioxidant role for phenolic degradation products from hydrolysis, microbial metabolism, or chemical degradation of cranberry flavonoids and polymeric components. Add to these also the numerous phenolic acids and hydroxycinnamic acids naturally present in the fruit, all of which are absorbed to a much greater extent than the antioxidant flavonoids that receive most attention, and the result could be significant antioxidant potential in vivo. Uptake, metabolism, and distribution of these quantitatively minor compounds have not been followed previously, but now are beginning to receive research attention as possible explanations for cranberry effects on cells and tissues, particularly the increases in antioxidant capacity and decreased biomarkers of oxidative damage observed following cranberry consumption.

Outcomes of cranberry antioxidant effects in vivo include reduced levels of oxidized LDL and AOPP that diminish cardiovascular health (Ruel et al., 2005; Ruel and Couillard, 2007), general reduction of oxidative stressors that lead to cell and tissue degeneration (Valentova et al., 2007), and signal transduction that affects systemic responses far downstream, as will be discussed in the Section titled Effects on Signal Transduction, Protein Expression, and Activity.

Indirect Antioxidant Activity

Increasing skepticism over the ability of dietary phenols to act as traditional antioxidants in vivo has emerged over the past several years. Cranberries and other fruits and vegetables have been shown to increase plasma antioxidant capacity (Pedersen et al., 2000), yet the concentrations of circulating flavonoids and polyphenols observed after their consumption remain very low (10–20 μM at most) and account for only a small percentage (2–4%) of total circulating antioxidants which include ascorbate, urate, and simple phenolics ($\sim$500 μM) (Stevenson and Hurst, 2007). Thus, cranberry flavonoids and polyphenols should have inconsequentially small effects as direct antioxidants, except for perhaps in the intestinal tract and digestive system. Alternative "indirect" antioxidant mechanisms must be examined to explain in vivo activity of cranberry polyphenols.

One indirect mechanism receiving considerable recent attention is induction of endogenous defense enzymes (Dragsted et al., 2004; Stevenson and Hurst, 2007). These include proteins directly responsible for countering oxidative stress, including superoxide dismutase, catalase, and glutathione peroxidase, those responsible for creating endogenous antioxidants, including γ-glutamylcysteine synthetase (GCS) and glutathione synthetase and those responsible for the metabolism of potentially reactive xenobiotics including glutathione S-transferases and quinine reductase, governed by electrophile-responsive element (EpRE) mediated enzyme expression (Dragsted et al., 2004; Stevenson and Hurst, 2007). This is an intriguing possible mechanism that could explain greatly increased plasma antioxidant capacity with only trace levels of absorbed polyphenols.

Both quercetin and bilberry extracts at low μM concentrations upregulated several EpRE containing genes in vitro, resulting in increased enzyme expression, suggesting promotion of phase II metabolic enzymes that counter electrophilic xenobiotics by generation of endogenous antioxidants and deactivation of toxic reactive oxygen species (Myhrstad et al., 2006). For example, GCS was significantly induced in COS-1 cells by low μM concentrations of quercetin ($\sim$3 fold) and kaempferol ($\sim$2 fold), but not myrcetin; this activity explained observations that quercetin (at 5 and 25 μM) significantly increased glutathione concentrations by as much as 50% (Myhrstad et al., 2002). In vivo, 27% and 45% cranberry juice supplementation for 120 days increased superoxide dismutase activity in the liver of orchidectomized rats ($p < 0.05$) (Deyhim et al., 2007). Such induction of defensive enzymes and endogenous antioxidant systems by flavonols has extensive ramifications for cancer prevention and cardiovascular disease.

While few reports of cranberry induction of protective proteins in vivo could be found, studies involving other fruits and vegetables may shed light on other potential mechanisms of antioxidant activity after cranberry consumption. In humans, six days of high fruit and vegetable diets did not affect antioxidant capacity measured by plasma FRAP and TEAC (which mostly measure electron transfers rather than traditional radical quenching), but they did significantly increase LDL oxidative lag time and induce glutathione peroxidase relative to control and vitamin/mineral-supplemented diets (Dragsted et al., 2004), demonstrating that consumption of fruits and vegetables increases enzymatic antioxidant defense mechanisms via nonnutrient mechanisms. Similar observations were made after consumption of apple juice and black currant juice (Young et al., 1999).

Although direct evidence is still limited, promotion of antioxidant-related enzymes or a similar mechanism is at least partially responsible for the strong inverse correlations between fruit and vegetable intake and cancer and other degenerative

diseases. Also likely is that promotion of antioxidant activity after consumption of cranberries is at least partially due to one or several of these enzyme-related mechanisms.

Effects on Signal Transduction, Protein Expression, and Activity

Recent evidence indicates that phytochemicals influence gene expression, enzyme activity, and signal cascades, and these actions may be responsible for much of the reported potential anti-cancer, anti-inflammatory, and neuro-protective effects of cranberries beyond the gastrointestinal tract in vivo. Although the molecular mechanisms involved have not yet been elucidated, it is likely that phenol covalent binding to proteins, e.g. through thiol groups, and subsequent proteolysis to signal peptides plays a critical role in these nutragenomic processes.

For example, cranberry phytochemicals may inhibit cancer by interfering with key pathways involved in cell function, most notably regulation of the cell cycle and growth via ornithine decarboxylase (ODC) inhibition, induction of apoptosis by unknown pathways, inhibition of invasion and metastasis via suppression of matrix metalloproteinases (MMP), and reduction of harmful inflammatory responses by modulating COX and NFk-B pathways (Neto, 2007a, 2007b). In these actions, the active cranberry components seem to be salicylic acid, ursolic acid and its derivatives, flavonols, and PACs. Isolated resveratrol also seems potently anticarcinogenic in vitro (Jang et al., 1997) and in vivo (Tessitore et al., 2000).

ODC, a regulator of polyamines that direct cell growth and proliferation, is often over-expressed in cancer cells (Kubota et al., 1995, 1997; Neto, 2007b). This over-expression contributes to cancer proliferation and invasion, especially in promoting mitogen activated protein kinase activity that seems crucial to cell proliferation and transformation (Kubota et al., 1995; Kubota et al., 1997). Limiting ODC expression and activity, then, is a potential means to control cancer development. An early in vitro study revealed that crude cranberry extracts and especially proanthocyanidin extracts were potent inhibitors of ODC activity in cancer cells (Bomser et al., 1996). Cranberry extracts also inhibited ODC production in mouse epidermal cells; the most active fraction containing primarily proanthocyanidins and flavonol glycosides inhibited ODC activity by 50% at 5.67 μg/mL (Kandil et al., 2002). Expression of ODC was also significantly reduced and ODC induction by lipopolysacharides was totally obliterated by crude cranberry polyphenol extracts (mg/mL levels) in a mouse fibroplast model (Matchett et al., 2005). This mechanism is probably most important in the mouth, stomach, and the digestive tract where absorption and bioavailability are not limiting issues, but binding to proteins on the intestinal mucosa surface may set off a signal cascade that alters systemic enzymes.

Cell cycle arrest and promotion of apoptosis are potent targets in halting the unchecked growth of cancerous tumors (Ramos, 2007). Various extracts of cranberry polyphenols promote apoptosis and cell cycle arrest in phases G_1 and G_2 at high (mg/mL) concentrations in breast cancer models (Ferguson et al., 2004; Sun and Hai Liu, 2006; Neto, 2007a, 2007b) and at low (μM) quercetin concentrations in liver cancer cells (HepG2) (Ramos et al., 2005), leading to the conclusion that in vitro anti-apoptotic activity of cranberries was due to this flavonol (Neto, 2007a; Neto, 2007b). In vivo, this mechanism may be active in the mouth, stomach, bladder, and digestive tract after cranberry consumption, but probably not otherwise, considering the relatively high concentrations necessary and the low bioavailability of flavonols.

Matrix metalloproteinases may facilitate alteration of the extracellular matrix, promoting invasion, proliferation, and metastasis (Pupa et al., 2002). Cranberry PACs, as well as PACs from other sources, inhibit MMP-2 and MMP-9 in cancer cells (Vayalil et al., 2004; Neto et al., 2006; Neto, 2007b). One cranberry PAC subfraction (characterized as mostly DP 4 to 7 with at least 1 A-type linkage) was particularly effective, inhibiting 90% of MMP-2 and MMP-9 in prostate tumor cells at 500 μg/mL in vitro (Neto et al., 2006). Ursolic acid and its cinnamic acid derivatives at 9 μM concentrations similarly inhibited MMP-2 and MMP-9 (Cha et al., 1996; Kondo et al., 2004; Neto, 2007b). In vivo, this mechanism may contribute to the inhibition of oral, stomach, and gastrointestinal cancers observed for cranberries.

Isolated resveratrol inhibits cancer at various stages in in vitro models, most notably by COX inhibition and induction of phase II metabolizing enzymes (Jang et al., 1997). In vivo, resveratrol's inhibition of precancerous lesions in colon cancer in rats was traced to modified expression of p21 and bax proteins regulating apopotosis and cell proliferation (Tessitore et al., 2000). However, the dosages (500 μg/kg) were very high, unlikely to be achievable from normal consumption of cranberry products, though perhaps possible by heavy supplementation.

Anti-inflammatory effects from cranberries may be related to reduced COX activity, NF-κB down-regulation, and inhibited adhesion molecule expression by diverse elements of the berry, including flavonols, anthocyanins, proanthocyanidins, hydroxycinnamic acids, resveratrol, and salicylic acid (Neto, 2007b; Ruel and Couillard, 2007). COX-1 and COX-2 are enzymes involved in the synthesis of pro-inflammatory prostaglandins from arachidonic acid. Aspirin (acetylsalicylic acid), a COX-1 inhibitor widely prescribed to control inflammation, irreversibly binds to the active site of COX-1 (Duthie et al., 2005). Trace levels (nM) of its parent, salicylic acid, inhibit COX-2 activity in vitro (Wu et al., 1991). After consumption of cranberry juice, plasma concentration of salicylic acid increase to levels high enough (high nM to low μM) to achieve this inhibition in vivo (Zhang and Zuo, 2004; Duthie et al., 2005).

COX-1 and COX-2 are also inhibited by cranberry anthocyanins, though only mildly ($\sim$10% reductions at 125 μg/mL) (Seeram et al., 2001). Crude cranberry polyphenol extracts and especially PAC-rich extracts show more pronounced effects, with 50% reductions of COX-1 activity at 170 μg/mL and 20 μg/mL, respectively (Neto, 2007b). Collectively, these data support COX inhibition and subsequent anti-inflammatory

action in the mouth and digestive tract following cranberry consumption due to polyphenols, and maybe more systemically due to salicylic acid. Whether cranberry phytochemicals influence COX expression in vivo is not yet known, but current research is investigating this possibility (Neto, 2007b).

The transcription factor, NF-κB, is a potent regulator of inflammation responses, affecting a wide variety of genes that code pro-inflammatory adhesion molecules, cytokines and chemokines (Ruel and Couillard, 2007). Quercetin at 50 μM and 100 μM (but not 5 μM) dose-dependently decreased NF-κB activation in rat hepatocytes induced by interleukin-1b (Martinez-Florez et al., 2005). In a rat model of colitis, anti-inflammatory effects of quercitrin were traced to attenuation of NF-κB and downstream effects on cytokines and inducible nitric oxide synthase (Comalada et al., 2005). Abation of inflammation thus provides yet another mechanism by which cranberries effectively influence pathologies in the mouth and digestive tract. Perhaps, after continual consumption of cranberry, the accumulation of cranberry flavonols in the urinary tract and bladder noted by Vorsa et al. (2007a) may also play some role in cranberries' prevention of urinary tract infections through anti-inflammation mediated by NF-κB.

Adhesion molecules are stimulated by cytokines and regulated by NF-κB. These play crucial roles in inflammation responses and atherosclerosis, linking leukocytes to endothelial cells and facilitating their absorption into inflamed tissues (Couffinhal et al., 1994). Numerous compounds found in cranberries, including proanthocyanidins, anthocyanidins, hydroxycinnamic acids, and salicylic acid, inhibit expression of adhesion molecules in vitro. A review by Ruel and Couillard (2007) reports previously unpublished observations of significantly lowered plasma levels of ICAM-1 and VCAM-1 in vivo as well, after 12 weeks of low calorie cranberry juice supplementation in obese men, providing yet another explanation for cardioprotective and anti-inflammatory effects from cranberry consumption.

Oxidized LDL (LDLox) have received considerable attention for their role in atherosclerosis. LDLox (Ruel et al., 2005) and AOPP (Valentova et al., 2007) are not only biomarkers of oxidative stress, but also play critical roles as signaling molecules that excite inflammatory responses in vivo. LDLox induces pro-inflammatory reactions from epithelial cells, mainly via NFκB activation (Robbesyn et al., 2004). AOPP promotes formation of pro-inflammatory cytokines, which in turn, activate immune cells (Kalousova et al., 2005; Valentova et al., 2007). As explained in Section titled Antioxidant Activities and Mechanisms, whatever cranberry phenols are absorbed inhibit LDL oxidation, probably by free radical scavenging. Thus, while the action of cranberry compounds may be indirect, the effect—lower circulating levels of LDLox and AOPP—directly and materially contributes to control of inflammation and, in turn, perhaps atherosclerosis (Ruel and Couillard, 2007; Valentova et al., 2007).

Finally, neurodegradation in animals may be influenced via protein expression and activity in addition to the protective antioxidant mechanisms usually attributed to cranberries (Lau et al., 2005). In a model of Alzheimer's disease, feeding blueberries to aged mice raised concentrations of extracellular signal regulated kinase (ERK) and protein kinase C (PKC), enzymes critical to cognitive functioning and neural signaling; controls without blueberries were unaffected (Micheau, 1999; Selcher et al., 1999). Levels of these proteins were later correlated with performance in motor tests of both young and aged rats (Lau et al., 2005 and references therein). It seems likely that comparable enzyme induction accounts also for the improved ex vivo neural signaling observed in aged, cranberry-fed rats (Shukitt-Hale et al., 2005). More research is needed to identify the active components of berries and the mechanisms by which the enzymes are induced.

EFFECTS OF PROCESSING, STORAGE, AND COMPOSITION ON CRANBERRY PHYTOCHEMICAL STABILITY

Because the characteristic high acidity and tart flavor of fresh cranberries are considered unpalatable by most consumers, cranberries are customarily processed into cranberry juice ($\sim$65%) and cranberry sauce ($\sim$30%), both of which are generally sweetened with sugars, other juices, or artificial sweeteners. Sweetened dried cranberries, the [often flavored] products of countercurrent cranberry juice extraction, are increasingly gaining popularity as well, though phytonutrient retention in this product has not been determined. Only about 5% of cranberries are sold in their fresh form, and the majority of these are probably not eaten raw, but rather used in prepared foods such as cranberry sauce or in baked goods. Whole cranberries have been noted for their excellent stability as compared to other fruits, and remain unspoiled for several weeks at room temperature if kept dry. In contrast, cranberry juice generally has a short shelf-life relative to other fruit juices, due to rapid deterioration of color quality, which is its most attractive and important quality factor (Francis, 1995). Loss of color, however, implies corresponding destruction of the responsible anthocyanins and for this reason, most research on processing and storage effects have focused on anthocyanins. Given the recent focus on health promotion through diet, understanding processing effects is critical not only for traditional goals of maintaining quality and prolonging the shelf life of cranberry products, but increasingly for conserving active phytochemicals that promote health effects as well.

Storage and Processing Factors Affecting Phytochemical Retention

Few published studies have examined the effects of storage on the composition of whole cranberries, and those have focused on anthocyanins. Because the color of the fruit does not accurately predict the color of the juice it produces, color

measurement of whole cranberries is rare (Francis, 1995). Rather, anthocyanin quantification predominates and is used as an indicator of the color quality of the final product. High levels of oxygen in controlled atmosphere storage have little effect on postharvest anthocyanin development and total phenolics (Gunes et al., 2002). In contrast, temperature significantly affects anthocyanins: storage at 15°C was optimal, as much as doubling anthocyanin content due to postharvest biosynthesis compared to storage at higher and lower temperatures (0, 5 or 20°C) (Wang and Stretch, 2001). Freeze-thaw treatments are widely used in the cranberry processing industry to increase anthocyanin yield to ∼50% (vs 7% in untreated fruit) as well as total juice yield (Sapers et al., 1983a). Cell wall deterioration due to ice crystal formation releases phytochemicals from normal cellular compartmentalization, resulting in higher extraction efficiency of anthocyanin pigments. It is likely that freeze-thaw treatments similarly release other classes of phytochemicals, such as the other flavonoids, but no experimental verification of this is available.

Processing variables such as heat, increased pH, light, dissolved oxygen, and ascorbic acid, and the presence of certain enzymes (polyphenol oxidases and glycosidases) markedly destabilize cranberry color and anthocyanin content, while pigment co-factors (including cinnamic acid derivatives and flavonoids) as well as certain metals (copper, iron, and tin) improve cranberry anthocyanin and color retention during storage (Francis and Servadio, 1963; Starr and Francis, 1973; Francis, 1995; Rein and Heinonen, 2004; Wrolstad et al., 2005; Starr and Francis, 1968). Phenolic condensation via several mechanisms seems likely to be a major mode of phytochemical loss during processing and storage in cranberry products.

Destabilizing Factors

Temperature may be the most important factor in cranberry anthocyanin stability, particularly during thermal pasteurization necessary for microbial stabilization but also during storage of cranberry products. Elevated temperatures not only accelerate the reactions already discussed, but also promote thermal degradation via other pathways. Solutions of anthocyanins at low pH (2–4) and high temperatures (100°C) degrade via hydrolysis of the sugar group, releasing highly unstable anthocyanin aglycones that quickly degrade to colorless products (Adams, 1973). Coumarin glycosides and benzoic acid derivatives have been identified as thermal degradation products of anthocyanins at lower temperatures, indicating pathways other than deglycosylation are also active (von Elbe and Schwartz, 1996).

Changes in the already low pH in cranberry juice products affect anthocyanins. Increasing instability of anthocyanins at higher pH is widely known and attributed to their conversion to equilibrium forms that are more sensitive to oxidation and subsequent degradation. In contrast, cranberry juice cocktail lowered to pH 2.2 retained ∼5% more total anthocyanins throughout twelve weeks of storage, compared to juice at its normal pH 2.7 (Starr and Francis, 1973).

Ascorbic acid, abundant in whole fresh cranberries, is lost in processed cranberry products due to exposure of the juice to heat, oxygen, enzymes, and metal ions in the juice during pressing, sterilizing, and bottling processes (Licciardello et al., 1952). Traditional thermal processing of cranberry products degrades ascorbic acid substantially (∼25%), so processors must add overages to achieve desired concentrations in the final product (Starr and Francis, 1968). Better conservation of this nutrient may prolong color stability and shelf life of cranberry products.

Paradoxically, pronounced destruction of anthocyanins results from ascorbic acid fortification of cranberry; this action is highly dependent on oxygen availability and probably proceeds via ascorbate oxidation with production of superoxide anions, hydrogen peroxide, and hydroxyl free radicals, all of which react with anthocyanins (Starr and Francis, 1973; Starr and Francis, 1968). Cranberry juice cocktail with only natural ascorbic acid levels (40 μg/mL) lost 53% of its anthocyanin content after 32 weeks, while juice fortified to 177 μg ascorbic acid/mL lost 63%; most notably, juice with added ascorbic acid (177 μg/mL), and also two mL of headspace oxygen lost 82% (Starr and Francis, 1968). Oxygen is also necessary for anthocyanin photosensitized degradation via singlet oxygen (Attoe and Elbe, 1981), it accelerates thermal degradation (Daravingas and Cain, 1968), and is a necessary substrate for browning reactions of polyphenol oxidase enzymes. Enzymes naturally present in berries, especially polyphenol oxidase and glycosidases, can wreak havoc on cranberry pigments and must be deactivated for acceptable product stability (Wrolstad et al., 2005; Rein, 2005).

The extensive oxidative polymerization that the diverse phenolic components of cranberries undergo during processing and storage is responsible for the formation of sediments in cranberry juice and, along with ascorbic acid oxidation, browning, and loss of color. Although browning reactions in cranberry juice have not been studied, the effects are recognized in widespread use of polymeric pigment assays as an index of degradation and polymerization of proanthocyanidin extracts during handling. Studied in other fruits, juices, wine, and model solutions, phenolic condensation and subsequent browning is initiated by oxidation mediated by enzymes, ascorbic acid degradation products, metal ions, and Maillard browning reaction products (Robards et al., 1999). Monophenols are oxidized to *o*-diphenols and then to quinones, which then may condense with other phenolic or nonphenolic species, eventually forming pigmented brown polymers (Robards et al., 1999). While phenolic condensation destroys native phenolics and flavonoids, it also may generate the high molecular weight NDM fraction of the juice. Whether the net effect of phenol condensations is increased health benefits from bioactive NDM or decreased activity via loss of more bioavailable low molecular weight phenols is an intriguing question, currently without answer. Tracking phenolic condensation in cranberry products as it relates to color loss, native flavonoid loss, and NDM formation should be feasible with more sensitive analytical techniques such as MALDI-MS and LC-MS.

Stabilizing Factors

Pigment co-factors (also known as copigments) can improve anthocyanin stability substantially, but they also alter spectral characteristics, shifting the wavelength maximum and increasing maximum absorption. The net effect of copigmentation is always to intensify the cranberry color and shift it towards bluer hues that show as magenta in the berries. After 103 days storage, whole cranberry juice retained only 20% of its original color intensity as measured by spectrophotometry, but juice with copigments added in tenfold molar excess relative to anthocyanins retained more color: 42% with added rosmarinic acid, 33% with sinapic acid, and 25% with ferulic acid over the same storage period (Rein, 2005). In model solutions, 3 mg/mL quercetin spared cranberry anthocyanins exposed to 150 μg/mL ascorbic acid by ~60% (Shrikhande and Francis, 1974). Copigmentation in cranberry juice appears to proceed through an intermolecular mechanism in which the pigment co-factor interacts with the anthocyanin through hydrophobic forces and/or hydrogen bonding to effectively "stack" or "sandwich" the anthocyanins, resulting in steric protection from nucleophilic attack (Rein, 2005). The flavonol quercetin also has a protective effect on anthocyanins, though whether it acts as a copigment or an antioxidant (or both) is unclear.

Overall, temperature and oxygen, both mediating multiple pathways of anthocyanin degradation, seem to be most important in cranberry anthocyanin stability and their interactions may be synergistic. Hence, anaerobic processing and packaging, nonthermal pasteurization, and use of pigment cofactors all seem promising approaches for controlling color and anthocyanin loss in cranberry products.

Effects of Processing and Storage on Phytochemical Composition

As noted above (Section titled Flavanols), in whole fresh cranberries anthocyanins are present at much higher levels than flavonols, but the reverse is true in cranberry juice. Compartmentalization of anthocyanin pigments in color bodies located in the fruit's skin impedes their release during pressing; whether similar compartmentalization exists for flavonols has not been reported, but seems less likely since flavonols are better retained in juice. Anthocyanins are also chemically less stable than flavonols, and are particularly sensitive to oxidation and light degradation during handling and processing. Anthocyanin interactions with ascorbic acid radicals may account for some degradation, but while general types of reactions that cause anthocyanin loss are recognized, specific reactions leading to loss of color and antioxidant capacity have not been elucidated.

Which sugar is attached to anthocyanins in cranberries plays a crucial role in their relative stability. Anthocyanin arabinosides are more easily degraded than galactosides under a wide range of conditions (varying levels of vitamin C, oxygen and light exposure) (Starr and Francis, 1968; Attoe and Elbe, 1981). Surprisingly, the nature of the anthocyanin backbone (whether peonidin or cyanidin) has little or no effect on color retention (Starr and Francis, 1968; Attoe and Elbe, 1981). The critical role of anthocyanins in cranberry color and health effects argues for the development of new approaches to retain and stabilize these phytonutrients during processing and storage of cranberry products.

While flavonol retention during cranberry juice extraction and processing appears to be markedly superior to that of anthocyanins, some flavonol degradation does occur. Flavonol stability has not been measured, but given known concentrations of flavonols in cranberries and commercially available juice (see Table 2), juice yields of ~0.8 liters per kilogram of berries, and 27% juice in cranberry juice cocktail, flavonol retention may be estimated at between 40% and 70%, compared to 2–30% for anthocyanins using the same calculations. Because flavonols stabilize anthocyanins and have important nutraceutical qualities of their own as well, new research tracing their degradation and modes of loss in cranberry products seems worthwhile.

For PACs, monomers, dimers, and trimers account for a higher proportion in commercial pressed cranberry juice than in fresh berries (Prior et al., 2001), while higher polymers decrease (Gu et al., 2003b). This pattern could result from PAC polymer binding in the pectin-cellulose berry matrix and hence impaired release during pressing, or from hydrolysis of higher PACs during pressing. Once in the juice, total PAC levels remain constant during commercial high temperature-short time pasteurization of juice, but polymerization occurs rapidly when extracts are handled at room temperature under laboratory lights (E. Pappas and K.M. Schaich, unpublished data). Thus, changes in the distribution of PAC molecular sizes may offer an interesting secondary marker (along with anthocyanin degradation) of juice mishandling and quality deterioration during processing and storage.

Information about processing and storage effects on other cranberry components (e.g. other phenolics and terpenes) is not available. However, some patterns of change may be inferred from comparisons of juice and fruit compositions. For example, in studies of freshly prepared versus commercially marketed cranberry juice, myricetin was better conserved than quercetin during processing and storage (Chen et al., 2001), but since the flavonol content in cranberries can vary greatly with variety, maturity, and other factors, it was not certain that these differences were due to processing and storage effects alone. Comparing commercially available juices to whole berries, benzoic and phenolic acids show variable processing stability (Zuo et al., 2002; Zhang and Zuo, 2004). For example, salicylic acid and its isomer *p*-hydroxybenzoic acid are present at comparable levels in whole fresh cranberries (23.2 μg/g and 21.6 μg/g respectively), but in commercially processed juice, salicylic acid was ~50 times more prevalent than its isomer (3.11 μg/mL and 0.07 μg/mL respectively). Conversions also occur: caffeic acid conjugates of quinic acid are found in processed cranberry juice, but have not been isolated from fresh juice or whole berries (Chen et al., 2001; Cunningham et al., 2004; Harnly et al., 2006).

At the present time, observations of compositional differences between whole berries and processed juice can mostly serve to pique interest in underlying causes; the data is inadequate to provide explanations, although some speculative explanations may be offered. As the health effects from cranberry consumption become more concretely linked to specific chemical components, it will become increasingly important to understand in detail how processing and storage alter key phytochemicals and to develop new processing approaches that optimize their conservation and maximize health benefits for consumers.

SUMMARY AND CONCLUSIONS

Combating infectious and degenerative disease through diet seemed completely implausible only about thirty years ago, but with mounting epidemiological, animal, and clinical data revealing the benefits of fruits and vegetable consumption, it is today among the hottest trends in food science and nutrition. Indeed, promotion and maintenance of health and prevention of disease via diet has gained acceptance across research and medical communities as well as among the general population. In this current climate, cranberry stands out as a unique dietary health promoter, with numerous potential and proven benefits. In particular, cranberries act as antioxidants, inhibitors of bacterial adhesion, and modifiers of protein activity and expression after consumption.

While prevention of UTIs in women is the most solidly documented dietary benefit of cranberries, further research will surely provide insights also into its other potential health effects. Among these, prevention of cancer and inflammation (especially in the mouth, stomach, digestive tract, and excretory organs), inhibition of the virulence of pathogens (especially in the mouth and stomach) and promotion of cardiovascular health remain quite promising. Foundations of research into the potential neuroprotection and anti-viral activities of cranberries have been laid, and these, too, offer exciting possibilities for use of cranberries in dietary interventions.

While the unique and plentiful A-type proanthocyanidins in cranberries have attracted much attention, recent findings regarding their lack of bioavailability (to the bladder and other tissues) cast doubt on their direct systemic activity. Unique metabolites resulting from their consumption may potentially explain the anti-uropathogenic effects of cranberries but none have yet been conclusively identified. Meanwhile, it seems that the health-promoting capacity of the diverse and almost unparalleled abundance of other phytochemicals (especially flavonols, salicylic acid, other simple phenolics, and benzoates) of cranberries has been largely overlooked. These and/or other still unidentified phytochemicals or metabolites may be important keys to the nutraceutical value of cranberries. Research into cranberry phytochemicals and their bioavailability will undoubtedly uncover exciting new information as the search for active components and underlying molecular mechanisms continues. As these links become clearer, it will be increasingly important for the food industry to understand the complex phytochemistry of cranberries and the many modes of degradation of health-promoting components and use this information to maximize the nutritional value of cranberry products for consumers. Key research gaps include the effects of processing and storage on flavonols, PACs, and smaller phenolics, as well as reaction details and mechanisms for degradation and modification of all cranberry components. Some approaches showing promise to achieve greater phytochemical retention and stabilization in cranberry juice include anaerobic processing, oxygen scavenging packaging, UV-absorbing packaging, and nonthermal or limited heat pasteurization technologies, including high pressure processing and ohmic heating.

In vivo and in vitro, many dietary effects of cranberries parallel those observed epidemiologically for fruits and vegetables in general, particularly regarding inhibition of cancer and cardiovascular disease. These associations provide strong support that cranberries share the healthfulness of its fellow fruits. The unique phytochemistry of cranberries and anti-pathogenic activity distinctly superior to other fruits and vegetables is compelling reason to eat more cranberries, especially for consumers at risk for bacterial infections. At the same time, other plant foods certainly provide nutrients and phytochemicals (and benefits stemming from them) that cranberries do not. It must be emphasized, therefore, that cranberry consumption is not the answer to the prevention of degenerative diseases and other conditions mentioned here. Rather, cranberries (and perhaps therapies deriving from its phytochemicals) are most beneficial when consumed as part of an otherwise healthy diet incorporating a variety of plant foods.

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