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Varietal Differences in the Quercetin, Kaempferol, and Myricetin Contents of Highbush Blueberry, Cranberry, and Thornless Blackberry Fruits

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Representative varieties of highbush blueberries, cranberries, and thornless blackberries were compared to determine the extent of differences in the distribution of quercetin, kaempferol, and myricetin. Quantitation was by HPLC using an external standard method. Fruits of four highbush blueberry varieties contained quercetin (24–29 mg/kg, fresh weight) but not kaempferol or myricetin. All three flavonols were found in cranberry fruits, the range for dark-colored fruit of six varieties being 112–250 mg/kg with quercetin, 11–24 mg/kg with myricetin, and 0–3 mg/kg with kaempferol. Within varieties, flavonol contents were smaller in the less highly pigmented berries. Ripe (black-colored) fruits of 12 thornless blackberry varieties and selections contained quercetin (5–35 mg/kg) and kaempferol (1–3 mg/kg). Flavonol and anthocyanin contents were smaller in less ripe fruits. No correlation was seen between the total anthocyanin content and the amounts of flavonols in these cultivars.

INTRODUCTION

The mutagenicity of quercetin, myricetin, and kaempferol, flavonols that are widely distributed in fruits and vegetables (Herrmann, 1976), has been demonstrated by the Ames test (Bjeldanes and Chang, 1977; Hardigree and Epler, 1978; MacGregor and Jurd, 1978) as well as in a mammalian system (Meltz and MacGregor, 1981). Some evidence for the carcinogenicity of mutagenic flavonols has been obtained by Pamukcu et al. (1980) and Hatcher et al. (1983), but this result could not be confirmed by Fukuoka et al. (1980), Morino et al. (1982), or Takanashi et al. (1983). Because of the possibility that the mutagenic flavonols may have adverse effects on human health, we have investigated varietal differences in their distribution in various vegetables known to contain these compounds (Bilyk et al., 1984; Bilyk and Sapers, 1985). In the present work, we examined samples of highbush blueberry (*Vaccinium corymbosum* L.), cranberry (*V. macrocarpon* Ait.), and thornless blackberry (*Rubus sp.*) cultivars for differences in the distribution of quercetin, myricetin, and kaempferol.

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EXPERIMENTAL SECTION

Blueberry and cranberry samples were obtained from the USDA, Rutgers University Blueberry and Cranberry Research Center in Chatsworth, NJ, in 1983. Ripe blueberry samples, weighing about 3 kg each, were harvested from six to eight bushes of four cultivars in early or mid July, depending on the earliness of the cultivar. The berries were cleaned, packaged in polyethylene freezing containers, and stored at −18 °C until analyzed. Single portions weighing 270–600 g (150–430 berries, depending on cultivar) were taken for flavonol extraction. Samples of six cranberry cultivars, weighing approximately 4 kg each, were harvested from several adjacent bogs at Chatsworth in late October. The berries were sorted into dark-, medium-, and light-colored subsamples, cleaned, packaged in polyethylene containers, and stored at −18 °C. A single portion of each dark- and medium-colored subsample, weighing 150–200 g (90–170 berries), was taken for flavonol extraction. Thornless blackberry samples were obtained from the USDA Agricultural Research Center in Beltsville, MD, in 1983. Ripe fruits were harvested from four to six bushes of each variety or selection in late July or early August, depending on the cultivar. The samples, weighing 1–2 kg, were cleaned, packaged in polyethylene containers, and frozen at −18 °C. During frozen storage, some of the blackberries in each sample turned red. [This

Table I. Quercetin Content of Ripe Highbush Blueberry Fruits

variety	SS/A[a]	TAcy[b]	quercetin,[c] mg/kg (fresh wt)
Earliblue	44.8	153	28.5 ± 0.9
Weymouth	13.2	129	26.1 ± 0.2
Coville	15.0	139	25.1 ± 0.1
Bluetta	22.4	135	24.0 ± 0.1

[a] SS/A = soluble solids (%)/titratable acidity (% citric acid). [b] TAcy = total anthocyanin (A_{543} × dilution factor/sample weight) (Sapers et al., 1984). [c] Mean for triplicate determination ± standard deviation.

characteristic of blackberries is indicative of a degree of heterogeneity in ripeness that cannot be detected visually in the fresh state. The less ripe fruits, which are more acidic, revert to a red color because of an intracellular pH change (Jennings and Carmichael, 1979)]. Therefore, after 1 month of storage, we sorted the blackberries in each frozen sample into red and black subsamples and analyzed the two groups separately. Single portions of these subsamples weighing between 100 and 240 g (30–60 fruits) were taken for flavonol extraction.

Flavonol isolation, purification, identification, and quantitation were performed according to procedures described previously (Bilyk et al. 1984; Bilyk and Sapers, 1985). Flavonol glycosides, extracted from the macerated berries with MeOH by a procedure that gave 98% recovery in experiments with spiked samples, were hydrolyzed and the aglycones determined quantitatively in triplicate by HPLC, using an external standard method. Flavonol identifications were based on comparisons of TLC R_f values and HPLC retention times with those of standards.

Flavonol distribution data for berry samples were compared with values of the soluble solids/acidity ratio (SS/A), an index of ripeness (Woodruff et al., 1960; Walsh et al., 1983), and total anthocyanin content (TAcy) obtained in parallel studies of the same samples reported separately (Sapers et al., 1984, 1985a, 1985b).

RESULTS AND DISCUSSION

Ripe samples of four highbush blueberry varieties contained small amounts of quercetin (24–29 mg/kg, fresh weight) (Table I). Kaempferol and myricetin could not be detected in any of the samples. These samples were similar in total anthocyanin content as well as in quercetin content. Starke and Herrmann (1976) found high levels of quercetin (105–159 mg/kg) as well as detectable levels of kaempferol and myricetin in several European varieties of bilberry (Heerma I and Heerma II), a related *Vaccinium* species.

Quantitative data on the distribution of quercetin, myricetin, and kaempferol in dark- and medium-colored samples of six cranberry varieties are given in Table II. Only Stevens, Early Black, and Ben Lear cranberries contained all three flavonols. The high concentration of quercetin in cranberry as compared to blueberry and blackberry is noteworthy. Variation among samples in flavonol content was large. It is not clear, however, whether these differences were entirely genetic. Environmental factors are known to have a large effect on the biosynthesis of anthocyanins in cranberries (Hall and Stark, 1972; Cracker, 1971); the anthocyanins and flavonols are closely related products of phenylpropanoid metabolism (Wong, 1976). Differences in flavonol content among the samples (dark- and medium-colored subsamples being compared separately) did not appear to be related to the anthocyanin content (Figure 1), correlations between these constituents lacking statistical significance. However, the data in Table II clearly indicate that the darker berries within a cranberry sample, which have a larger total anthocyanin concentration, contained more quercetin and myricetin than the medium-colored samples, this effect being highly significant by analysis of variance (ANOVA) ($p < 0.0001$). The former samples were slightly more ripe than the latter, based on their greater soluble solids/acidity ratios (Sapers et al., 1985b).

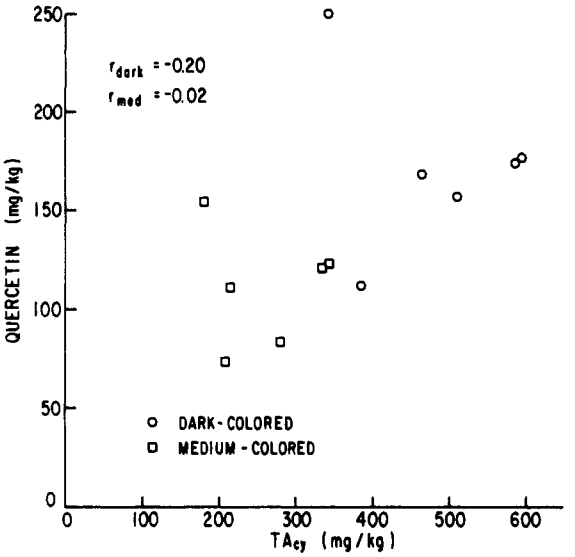

Figure 1. Relationship between quercetin and total anthocyanin contents in dark-colored (○) and medium-colored (□) fruits of six cranberry varieties.

Puski and Francis (1967) isolated flavonol glycosides from cranberries and identified their aglycons as quercetin and myricetin by chromatographic and spectral analyses. Using an electrophoretic method, Cansfield and Francis

Table II. Quercetin, Myricetin, and Kaempferol Contents in Different Varieties of Cranberry Fruits

variety	color	SS/A[a]	TAcy,[b] mg/kg	flavonol content,[c] mg/kg (fresh wt)		
				quercetin	myricetin	kaempferol
Stevens	dark	4.2	342	250.0 ± 0.2	15.8 ± 0.2	2.1 ± 0.5
	medium	3.9	180	153.7 ± 0.4	9.1 ± 0.3	2.4 ± 0.1
Early Black	dark	4.3	596	177.4 ± 1.5	17.3 ± 0.2	1.8 ± 0.2
	medium	4.0	335	122.1 ± 0.7	10.2 ± 0.2	1.8 ± 0.1
Ben Lear	dark	4.6	510	157.3 ± 0.6	16.8 ± 0.1	2.7 ± 0.1
	medium	4.2	279	82.9 ± 2.2	4.0 ± 0.1	1.1 ± 0.1
Franklin	dark	4.8	586	174.5 ± 1.1	10.8 ± 0.1	ND[d]
	medium	4.5	343	123.1 ± 0.9	7.0 ± 0.1	ND
McFarlin	dark	4.4	463	169.0 ± 1.5	23.7 ± 0.1	ND
	medium	4.2	213	111.4 ± 3.0	13.4 ± 0.2	ND
Howes	dark	4.7	386	112.4 ± 2.5	26.7 ± 1.1	ND
	medium	4.4	208	73.0 ± 1.7	14.3 ± 0.8	ND

[a] SS/A = soluble solids (%)/titratable acidity (% citric acid). [b] From Sapers et al. (1985b). [c] Mean for triplicate determination ± standard deviation. [d] ND = not detectable.

Table III. Quercetin and Kaempferol Contents in Different Varieties of Thornless Blackberry Fruits

variety or selection	color	SS/A[a]	TAcy[b] 1982	TAcy[b] 1983	flavonol content,[c] mg/kg (fresh wt) quercetin	flavonol content,[c] mg/kg (fresh wt) kaempferol
varieties						
Smoothstem	black	21.7	124	144	19.4 ± 0.1	2.1 ± 0.1
	red	7.1	84	93	12.1 ± 0.7	0.6 ± 0.0
Black Satin	black	11.1	114	113	12.8 ± 0.1	0.9 ± 0.1
	red	4.9	77	46	7.9 ± 0.4	0.4 ± 0.0
Dirksen Thornless	black	20.3	118	142	9.7 ± 0.7	0.9 ± 0.3
	red	6.6	81	74	8.1 ± 0.7	0.1 ± 0.0
Hull Thornless	black	24.2	75	63	7.3 ± 0.8	1.2 ± 0.0
	red	7.3	51	49	2.0 ± 0.1	0.7 ± 0.0
Thornfree	red	6.6	91	81	13.2 ± 0.2	0.6 ± 0.1
selections						
C-33	black	27.6	120	121	35.4 ± 1.2	2.6 ± 0.2
	red	6.6	99	84	12.8 ± 0.1	0.9 ± 0.1
C-55	black	15.7	111	107	18.1 ± 0.5	2.6 ± 0.0
	red	5.2	67	71	14.0 ± 0.1	1.6 ± 0.0
C-60	black	19.4	120	116	8.3 ± 0.1	1.9 ± 0.0
	red	6.8	96	84	6.2 ± 0.3	0.6 ± 0.0
C-57	black	13.3	130	124	8.2 ± 0.2	2.2 ± 0.1
C-62	black	19.1	70	81	5.2 ± 0.1	0.6 ± 0.0
C-58	red	4.8		63	16.8 ± 0.6	2.2 ± 0.1
C-52	red	7.3	56	63	11.0 ± 0.1	0.5 ± 0.0

[a] SS/A = soluble solids (%)/titratable acidity (% citric acid). [b] TAcy = total anthocyanin (A_{543} × dilution factor/sample weight) (Sapers et al., 1985a). [c] Mean for triplicate determination ± standard deviation.

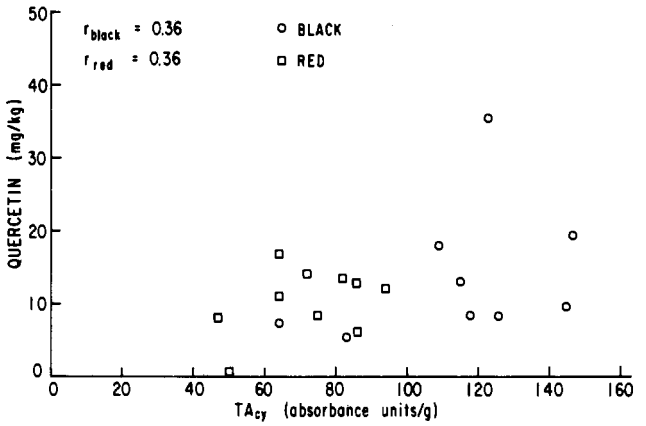

Figure 2. Relationship between quercetin and total anthocyanin contents in black- and red-colored fruits of 12 thornless blackberry cultivars.

(1970) found quercetin and myricetin but no kaempferol in an extract of Early Black cranberries. Their methodology provided only qualitative data.

The results of our comparison of different varieties and selections of thornless blackberries are summarized in Table III. The analytical data show differences in the total anthocyanin, quercetin, and kaempferol contents between black and red subsamples of each variety or selection, values being smaller in the less ripe (smaller SS/A) red fruits. (This color effect also was highly significant by ANOVA, i.e., $p < 0.0001$.) The quercetin content of black fruits ranged between 7 and 19 mg/kg (fresh weight) for four of the commercial varieties and between 5 and 35 mg quercetin/kg (fresh weight) for five of the blackberry selections. Too few black-colored fruits were obtained from the frozen Thornfree, C-52, and C-58 samples to permit analysis for flavonols. The kaempferol content in these samples constituted about 1–3 mg/kg (fresh weight). Myricetin was not detected in thornless blackberry samples. Henning (1981) reported the presence of quercetin and kaempferol glycosides at 10–100 ppm levels in five cultivars of blackberries. Some concentration differences in specific flavonol glycosides were evident among the cultivars he investigated.

The degree to which environmental factors such as soil and weather influence the flavonol content of blackberries is not known. However, we have observed relatively little variability in the total anthocyanin content of these cultivars for samples taken from the same location over two successive seasons (Table III).

Comparing the contents of quercetin and kaempferol to the total anthocyanin contents of these samples (red- and black-colored subsamples being compared separately), we saw no relationship between the ability of blackberry varieties to accumulate anthocyanin and their ability to accumulate flavonols (Figure 2). Correlations between the flavonol and total anthocyanin contents were not statistically significant. This finding also was reported by Starke and Herrmann (1976). We suggest that the relationship between flavonol accumulation and anthocyanin accumulation in representative cultivars of various fruits be given further study, excluding environmental influences insofar as possible. The lack of a correlation between these constituents might be helpful to breeders if they should be required to select for reduced flavonol content without also decreasing total anthocyanin content, which largely determines fruit color.

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Registry No. Quercetin, 117-39-5; kaempferol, 520-18-3; myricetin, 529-44-2.

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Tin, Iron, and Aluminum Contents of Commercially Canned Single-Strength Grapefruit Juice Stored at Varying Temperatures

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The extent of corrosion of commercially canned single-strength grapefruit juice was related to storage time and temperature. Storage of juices over a 12-week period at temperatures ranging from 10 to 50 °C resulted in increased levels of tin, iron, and aluminum in the product. The Sn, Fe, and Al uptakes were essentially linear with time, increased with temperature, and were greater for Sn than for Fe or Al. Corrosion of cans was disproportionally higher at temperatures exceeding ca. 37 °C. An Arrhenius plot of log K (Sn uptake) vs. the reciprocal of absolute storage temperature showed two distinct reaction kinetics. For the region 10–37 °C, an energy of activation, E_a, of 10.1 kcal and a Q_{10} of 1.8 was noted, whereas the region 37–50 °C showed an E_a of 20.2 kcal and a Q_{10} of 2.6. Examination of individual cans for their contents of Sn, Fe, and Al by regression analysis yielded linear correlation coefficients of 0.809 (Sn vs. Fe), 0.791 (Sn vs. Al), and 0.995 (Fe vs. Al).

The corrosiveness of liquid foods packaged in tin plate containers varies considerably, and in canned acidic foods corrosion is influenced by a number of chemical and physical parameters. Studies on canned citrus juices have identified the following corrosion-related factors: pH (Rouseff and Ting, 1985), corrosion accelerators such as sulfites and sulfur dioxide (Saguy et al., 1973), oxygen (Kefford et al., 1959), high storage temperature (Nagy et al., 1980; Rouseff and Ting, 1985), and processing variables such as deaeration, improper cooling, and increased headspace (Bakal and Mannheim, 1966).

During storage of canned citrus juices, an interaction occurs between the components of the juice and compositional material of the can. In plain tin cans (unlacquered), cathodic protection of the steel base is the result of the tin coating functioning as the sacrificial anode. Tin dissolution follows three well-defined stages (Saguy et al., 1973): (1) oil and tin oxide layers are removed from the can surface and the rate of tin dissolution is high; (2) continued dissolution of the tin causes enlargement of existing pores and scratches exposing the steel and the alloy; the corrosion rate is almost constant; (3) large areas of steel are exposed and there is a high dissolution rate of tin and iron; hydrogen evolves at a fast rate causing loss of vacuum and possible swelling.

An increase in the tin content of canned citrus juices during storage is inevitable. Two advantages of citrus juices packed in cans are that the tin plate minimizes browning development and vitamin C loss (tin functions as an antioxidant). However, disadvantages of canned juices are that elevated tin levels impart a metallic off-flavor (commonly referred to as tinny off-flavor), and if excessive amounts of tin dissolve (>400 ppm) and are ingested, nausea, vomiting, diarrhea, fever, and headache may result (Omori et al., 1955). Additionally, more recent studies with rats have indicated that excessive levels of tin in the diet might cause reduced retention of calcium, copper, and zinc in tissues (Yamaguchi et al., 1980; Greger and Johnson, 1981; Johnson and Greger, 1982). Attempts to reduce the tin content through use of lacquered cans have produced juices with poorer vitamin C retention (Curl, 1949) and significant discoloration (Mannheim and Hoenig, 1971).

Extensive studies on corrosion of canned citrus products by our laboratory (Rouseff and Ting, 1980, 1985; Nagy et al., 1980; Nagy and Rouseff, 1981) prompted us to undertake an extensive study on the effects of storage conditions (time–temperature) on the accumulation of tin,

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