

## Flavonoid Content of U.S. Fruits, Vegetables, and Nuts

JAMES M. HARNLY,<sup>\*,†</sup> ROBERT F. DOHERTY,<sup>†</sup> GARY R. BEECHER,<sup>†</sup>  
JOANNE M. HOLDEN,<sup>§</sup> DAVID B. HAYTOWITZ,<sup>§</sup> SEEMA BHAGWAT,<sup>§</sup> AND  
SUSAN GEBHARDT<sup>§</sup>

Food Composition Laboratory and Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

Analytical data are reported for 20 flavonoids (as aglycones) determined for more than 60 fresh fruits, vegetables, and nuts collected from four regions across the United States at two times of the year. Sample collection was designed and implemented by the Nutrient Data Laboratory (USDA). Analyses of eight flavan-3-ols (catechin, catechin gallate, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallic catechin, and gallic catechin gallate), six anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin), two flavanones (hesperetin and naringenin), two flavones (apigenin and luteolin), and two flavonols (myricetin and quercetin) were performed by the Food Composition Laboratory (USDA) using a hydrolysis method for the anthocyanidins, flavones, and flavonols and a direct extraction method for the flavan-3-ols and flavanones. Experimental results compare favorably (few statistically significant differences) to literature values in the flavonoid and proanthocyanidin database previously compiled by the Nutrient Data Laboratory. The results of this study showed a seasonal variation only for blueberries. This study also showed that the variation in the flavonoid content of foods, as purchased by the U.S. consumer, is very large. The relative standard deviation, averaged for each flavonoid in each food, was 168%.

**KEYWORDS:** Flavonoids; fruits; vegetables; nuts; aglycones; seasonal variation

### INTRODUCTION

There has been considerable interest in the flavonoid content of foods since the early 1980s when the studies of Steinmetz and Potter (*1*) demonstrated a relationship between a diet high in fruits and vegetables and a reduced risk of chronic diseases. Because reduced risk did not correlate with traditional nutrients, attention has focused on many non-nutrient, potentially bioactive compounds, of which the flavonoids constitute one family (*2*). Flavonoids are polyphenolic compounds with a C6–C3–C6 backbone. They can be subdivided into five structural categories: flavones, flavonols, flavanones, flavan-3-ols (catechins), and anthocyanidins. These compounds (aglycones) are commonly glycosylated (at one or more sites with a variety of sugars) and may also be alkoxyated or esterified. As a result, over 5000 different flavonoids have been identified in plant materials (*3*).

Research on the health impact of flavonoids requires a database that provides quantitative information on specific compounds in specific foods. A flavonoid database (FDB) was established in 2003 and a proanthocyanidin database (PDB) was established in 2004 by the Nutrient Data Laboratory at USDA (*4*). The FDB is based on a survey of literature data from national and international studies, whereas the PDB is based

primarily on experimental results from the Arkansas Children's Nutrition Center (*5–7*). The data quality for each source included in the FDB was evaluated using five criteria (sampling plan, number of samples, sample handling, analytical method, and analytical quality control) (*8*). In general, the data from each source were for a limited number of compounds for locally collected samples and cultivars. There are significant gaps in the FDB with respect to foods and specific flavonoids. The lack of comprehensive data is due to the large number of foods that contain flavonoids, the large number of glycosylated flavonoids, and the lack of analytical standards for most of these glycosylated compounds.

A comprehensive survey of flavonoids in U.S. foods requires a valid national sampling plan and analytical methods that can identify and quantify flavonoids (aglycones and glycosylated) in all five structural categories. To support the National Food and Nutrient Analysis Program, the Nutrient Data Laboratory and National Agricultural Statistic Service of the USDA (Beltsville, MD) developed statistically valid sampling protocols based on market data for a variety of foods (*9, 10*). These protocols call for the collection of samples as the average consumer would purchase them and ensures that the analytical results are representative of the food supply.

A large number of methods have been reported for the determination of flavonoids. In general, they were used either to determine flavonoids in a single category for a variety of

<sup>†</sup> Food Composition Laboratory.

<sup>§</sup> Nutrient Data Laboratory.

foods or to determine all of the flavonoids in a single food. Only two papers have described methods designed to cover all five categories of flavonoids (11, 12). In each case, quantification was achieved by hydrolyzing the glycosylated flavonoids to allow comparison to available aglycone standards. Merken and Beecher (11) described a method for the separation of 17 aglycones representing all five categories of flavonoids. The flavonoids were simultaneously extracted and hydrolyzed to produce the aglycones by refluxing the samples in an acidified methanol solution. The aglycones were then separated by high-performance liquid chromatography (HPLC) with diode array detection. Hydrolysis to produce aglycones served multiple purposes: it reduced the number of compounds and made chromatographic separation easier to achieve; it permitted quantification of flavonoids because standards for a large number of the glycosylated flavonoids are not available; and it provided data consistent with the earlier view that flavonoids were absorbed only in the intestine as aglycones. Unfortunately, hydrolysis also leads to degradation of the aglycones. A pseudo-first-order kinetics method was used for the quantification of flavones, flavonols, and anthocyanidins (13). The degradation of the flavanones and flavan-3-ols was too rapid for the application of the kinetics method. A separate extraction procedure (90% methanol without hydrolysis) followed by the same separation and detection procedure was used to determine these compounds.

Sakakibara et al. (12) described a method for the determination of "all" flavonoids in vegetables, fruits, and teas. They also identified isoflavones, anthraquinones, chalcones, and theaflavins. Their method was similar to that of Merken and Beecher (11), using a 90% methanol extraction, separation by HPLC, and diode array detection. Extracts of the samples were separated and the glycosylated flavonoids identified. The extracts were then hydrolyzed and separated, and the aglycones were identified and quantified. Thus, glycosylated flavonoids were identified, but quantitative results were based on the aglycones. They obtained recoveries of 68–92% for added flavonoids, and the analytical precisions ranged from 1 to 9%.

The present study reports quantitative results for 21 prominent flavonoids (as aglycones) for more than 60 fresh fruits, vegetables, and nuts collected in a market study across the United States. This project was a collaboration between the Food Composition Laboratory and the Nutrient Data Laboratory at USDA with financial support from the National Institutes of Health and the Produce for Better Health Foundation. The foods to be analyzed were selected on the basis of their high consumption, a lack of data, and their expected flavonoid content. Samples were collected directly from the marketplace according to the sampling protocols designed by the Nutrient Data Laboratory (9, 10) and were analyzed by the Food Composition Laboratory using the method of Merken and Beecher (11).

## MATERIALS AND METHODS

**Chemicals.** Myricetin and spectrophotometric grade trifluoroacetic acid (TFA) were purchased from Aldrich Chemical (Milwaukee, WI). *tert*-Butylhydroquinone (TBHQ) was purchased from Eastman Chemical Products, Inc. (Kingsport, TN). Apigenin, (+)-catechin gallate, cyanidin chloride, delphinidin chloride, (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin, (–)-epigallocatechin gallate, (+)-gallic acid, (+)-gallic acid gallate, luteolin, malvidin chloride, pelargonidin chloride, and peonidin chloride were purchased from Indofine Chemical Co. (Somerville, NJ). Petunidin chloride was purchased from Polyphenols AS (Sandnes, Norway). (+)-Catechin hydrate, (+)-gallic acid gallate, hesperidin, hesperetin, naringin, naringenin, narirutin, and quercetin were purchased

from Sigma (St. Louis, MO). Hydrochloric acid, HPLC-grade acetonitrile, and methanol were purchased from Fisher Chemical (Fair Lawn, NJ). High-purity water (18 M $\Omega$ ) was prepared using a Milli-Q purification system (Millipore Corp., New Bedford, MA).

All chemicals were maintained in a desiccator at –80 °C for the duration of the study. When stock standard solutions were prepared, crystalline standards were brought to room temperature under desiccation, quickly weighed under low-humidity conditions, and immediately returned to the desiccator and freezer. Prepared stock standard solutions were subjected to HPLC analysis using the same program as for food flavonoid quantification. Each chromatogram was carefully scrutinized for extraneous peaks, and the full absorbance spectrum (200–660 nm) for each flavonoid standard peak was carefully examined. If even small amounts of contaminants appeared, the stock standard solution and the crystalline standard were rejected, and a new source of that flavonoid standard was requisitioned until a "pure" standard was obtained.

**Food Samples.** The primary criteria for the selection of a food for flavonoid analysis included (a) fruits and vegetables that are highly consumed in the United States and for which there were only limited or no data; (b) fruits and vegetables that are highly colored, expected to contain flavonoids but for which composition data were sparse or lacking; and (c) nuts commonly consumed in the United States purported to have health benefits and for which there was a dearth of data relative to their flavonoid content.

The sampling protocols have been previously described (9, 10). Briefly, fresh samples of over 60 foods were collected from retail outlets in 12 generalized consolidated metropolitan statistical areas selected proportional to population size based on adjusted 1990 U.S. Census data. Samples were collected from three pickup locations in each of four national regions. Composite samples were prepared from the three locations of each region. In most cases, the pickups from the same locations were repeated approximately 6 months later. This approach was designed to ensure that analytical results are representative of the food supply, incorporating samples reflecting seasonal variation as well as imported samples available at different times of the year.

Samples were frozen upon collection and later freeze-dried, ground, and composited by region. The exceptions were nuts and dried fruits. These were not frozen or freeze-dried before grinding and compositing. The result was eight samples for each food: four regional composites collected twice during the year (2 passes). Sample pick-up, shipping, and processing were performed by organizations under contract to the Nutrient Data Laboratory. Freeze-dried powdered samples were shipped to the Food Composition Laboratory. For a limited number of foods (artichokes, broccoli, and potatoes), cooked, as well as raw, samples were analyzed. Cooking was performed after collection by the contract organization (14). The cooked samples were then composited by region and frozen.

**Sample Preparation. Hydrolysis.** The hydrolysis procedure has been described previously (11). Briefly, freeze-dried powdered samples (0.5–7.0 g, depending on the level of the flavonoids and the availability of the sample) were refluxed at 75 °C for 5 h in 50 mL of acidified methanol (1.2 N HCl) with 0.4 g/L TBHQ. Every 0.5 h, a 2 mL aliquot was removed, cooled, sonicated, filtered, and placed in an HPLC sampling vial.

**Direct Extraction.** Freeze-dried powdered samples (0.2–0.5 g) were homogenized for 3 min in a tissue homogenizer with 4 mL of 90% aqueous methanol with 0.4 g/L TBHQ. Samples were then centrifuged, and the solvent was removed. Fresh solvent was added to the solid, the homogenization repeated, and the solvent removed and combined with the first supernate. This step was repeated four times or more, until the solvent was clear. The combined extraction volume was reduced to less than 1 mL by purging with N<sub>2</sub> and then brought to a volume of 1 mL. Samples were then filtered and placed in autosampler vials.

**HPLC Instrumentation.** An Agilent Series 1100 (Wilmington, DE) HPLC was used for this work with a Zorbax Eclipse XDB-C18 column (250 × 4.6 mm, 5  $\mu$ m) and a guard column (12.5 × 4.6 mm) of the same stationary phase. Both were thermostated at 30 °C with a flow rate of 1.0 mL/min. The sample injection volume was 5  $\mu$ L. The diode array detector acquired spectra for the full range with specific

**Table 1.** Calibration Information

flavonoid	method <sup>a</sup>	wave-length <sup>b</sup> (nm)	sensitivity <sup>b</sup> (mAU-s/ g/mL)	detection limit <sup>c</sup> (g/mL) <sup>b</sup>	detection limit <sup>d</sup> (mg/100 g) <sup>b</sup>
flavan-3-ols <sup>e</sup>					
C	DE	210	174	3	0.06
CG	DE	210	184	3	0.05
EC	DE	210	197	3	0.05
ECG	DE	210	184	3	0.05
EG <sup>e</sup>	DE	210	194	3	0.05
EGCG	DE	210	195	3	0.05
GC	DE	210	159	3	0.06
GCG	DE	210	184	3	0.05
anthocyanidins					
cyanidin	HYD	520	92	5	0.4
delphinidin	HYD	520	89	6	0.4
malvidin	HYD	210	93	5	0.4
pelargonidin	HYD	520	75	7	0.5
peonidin	HYD	520	103	5	0.4
petunidin	HYD	520	64	8	0.6
flavanones					
hesperetin	DE	210	83	6	0.1
hesperidin	DE	210	63	8	0.2
naringenin	DE	210	101	5	0.1
naringin	DE	210	50	10	0.2
naringin	DE	210	50	10	0.2
flavones					
apigenin	HYD	210	126	4	0.3
luteolin	HYD	210	32	16	1.2
flavonols					
kaempferol	HYD	210	23	22	1.8
myricetin	HYD	210	114	4	0.4
quercetin	HYD	210	81	6	0.5

<sup>a</sup> Direct extraction (DE) or hydrolysis (HYD). See Materials and Methods. <sup>b</sup> Units: mAU-s/ $\mu$ g/mL = milliabsorbance units per microgram per gram of standard; g/mL = micrograms per milliliter; mg/100 g = milligrams per 100 grams of sample, fresh weight. <sup>c</sup> Detection limits for calibration curve. Concentration that gave integrated absorbance of approximately 500 mAU-s ( $\sim 3\sigma$ ). <sup>d</sup> Detection limits for fresh samples based on 90% moisture content and either 0.5 g (DE) or 5.0 g (HYD) sample sizes. <sup>e</sup> Abbreviations: C, catechin; CG, catechin gallate; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin gallate; EGCG, epigallocatechin gallate; GC, galocatechin; GCG, galocatechin gallate.

monitoring at 210, 260, 278, 370, and 520 nm. The solvents were (A) methanol, (B) acetonitrile, and (C) trifluoroacetic acid. Over the 60 min run, the concentration ratios for A/B/C varied linearly from 90:6:4 at 0 min, to 85:9:6 at 5 min, to 71:17.4:11.6 at 30 min, and to 0:85:15 at 60 min.

**Calibration Standards and Detection Limits.** Unlike carotenoids, retinoids, and tocopherols, highly accurate, commonly accepted, and widely publicized extinction coefficients at specific wavelength(s) and for specific solvent(s) are not available for food-containing flavonoids. Although there may be a few such values for a very limited number of flavonoids, the accuracy of these values is subject to question. In lieu of the lack of these data, flavonoid standards were purchased from commercial sources. Standards were kept in a desiccator at  $-80^\circ\text{C}$  conditions (see Chemicals).

Calibration curves were produced by appropriate serial dilution of the stock standard materials listed above. Worksheet templates were prepared in Microsoft Excel (Redmond, WA) for each flavonoid. Following preparation of new standards or maintenance on the HPLC, analytical sensitivity was checked to ensure the validity of the calibration curves and templates. Detection limits varied with individual flavonoids (different sensitivities led to different detection limits in terms of micrograms per milliliter) and individual samples (different sample masses and moisture content led to different detection limits in terms of micrograms per gram). Rather than numerical detection limits, "not detected" was recorded in the log books. For the data tables in this study, "not detected" has been translated to "0.0". If samples were not analyzed, there is no entry. **Table 1** provides a list of each flavonoid aglycone, the method of analysis (hydrolysis or direct extraction), the

wavelength used for detection, the sensitivity of the calibration curve, and the detection limit of the calibration curve in grams per milliliter and the detection limit in milligrams per 100 g based on a moisture content of 90% and either a 0.5 g sample (direct extraction) or a 5 g sample (hydrolysis).

**Flavonoid Identification.** For all of the flavonoid subclasses except anthocyanidins, 210 nm was the wavelength chosen for monitoring the chromatograms and quantification of data. Absorbance at 210 nm was selected because it gave substantially more sensitivity and therefore lower limits of detection than the traditional wavelengths of maximum absorption for each of the flavonoids (260 nm for flavones, 278 nm for flavanones and flavan-3-ols, and 370 nm for flavonols). Anthocyanidins, with the exception of malvidin, were monitored at the traditional 520 nm. The sensitivity and detection limits for malvidin were better at 210 nm than at 520 nm. Absorbance at 210 nm is nonspecific and therefore offers the possibility that compounds other than flavonoids may coelute and bias the data. However, this is also true at the traditional wavelengths, although to a somewhat lesser extent. Regardless of the wavelength monitored by the chromatogram, accurate identification must be based on the complete absorption spectrum (200–600 nm). For every potential flavonoid peak, the complete absorption spectrum was visually evaluated and compared to that of the appropriate pure standard using the "purity index" value calculated by the Agilent software. This is a cross-correlation calculation that evaluates the similarity of the spectra. If there was any indication of contamination at 210 nm (they were minimal), then the traditional wavelength was employed for quantification of the flavonoid.

**Kinetic Calculations.** Absorbance values for each flavonoid peak were converted to concentration using the appropriate calibration curve. The concentrations for the 10 aliquots collected from the hydrolysis procedure (one sample every 30 min for 5 h) were entered into a template prepared in Microsoft Excel (13). The extrapolated values were entered into a spreadsheet that contained the sample weight and moisture content to provide the final concentration in terms of milligrams per 100 g of fresh weight.

**Quality Control.** Commercial standards were checked for purity prior to dilution for calibration standards (as stated earlier) and cross-checked with standards from alternate sources to verify accuracy. The only available Standard Reference Material with values for flavonoids is baking chocolate (SRM 2384), which is certified for (+)-catechin and (–)-epicatechin. Analysis of this material yielded recoveries within the confidence limit.

New calibration standards were checked against preceding standards. Flavonoid standards of graded concentrations were separated on the HPLC system periodically during these analyses. "Standard" response lines were calculated from peak area data, compared to earlier lines, and adjusted when appropriate for such factors as column age, minor alterations in solvents, and changes in detector light sources. Tables were developed for retention times and UV–vis spectra recorded by the diode array detector. Templates were developed in Microsoft Excel for calibration and for the pseudo-first-order kinetics method. The absorbance spectra of all peaks were compared to reference spectra of pure standards using the matching subroutine of the Chemstation software (Agilent, Wilmington, DE) to verify the accuracy of the peak identification. In cases of doubt, samples were spiked with flavonoid standards to verify identification.

An in-house blueberry control material was developed and analyzed at routine intervals to monitor the repeatability of the hydrolysis process. Blueberries were chosen because of their high content of the very labile anthocyanidins. Consideration was given to the preparation of a "mixed" food QC material but was discounted because of the possible destructive interaction of organic acids (from citrus) with flavonoids during and after homogenization.

Individually quick-frozen blueberries were pulverized to pass 60 mesh sieves at the National Institute of Standards and Technology's Cryogenic Homogenization Facility. The homogenized material was thoroughly mixed, transferred to 4 oz brown glass bottles, flushed with nitrogen, capped, and stored at  $-80^\circ\text{F}$  for the duration of the project. Ten bottles were randomly selected and sampled to validate homogeneity on the basis of anthocyanidin analysis. The between-bottle relative standard deviation (RSD) for each anthocyanidin (cyanidin, 8%;

delphinidin, 7%; malvidin, 6%; and peonidin, 7%) was not significantly different from the within-bottle RSD, indicating homogeneity of the blueberry control material.

**Statistical Calculations.** Final compilation of the data and all *t* test calculations were performed using SAS 9.1 (SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

**Analytical Results.** A summary of the results of this study is reported in **Tables 2, 3, and 4** for fruits, vegetables, and nuts, respectively, in the rows labeled FCL. The mean, standard deviation, and the number of regional samples analyzed are listed. The samples are identified by the name used in the USDA National Nutrient Database for Standard Reference and the national nutrient databank number (NNDB No). Values are reported for eight flavan-3-ols, six anthocyanidins, two flavanones, two flavones, and three flavonols for more than 60 different foods. Specific cultivar information is provided for apples, kiwis, plums, broccoli, lettuce, and potatoes. If flavonoids were not detected (the concentration was less than the limit of detection), a value of 0.0 has been listed.

On average, five of the eight regional samples were analyzed for each food. It was not possible to analyze all eight samples in every case because of the time demands of the analytical method. For each food, at least one sample was analyzed for each pass. Further analyses were based on the levels of flavonoids found. In general, four or more regional samples were analyzed for 80% of the foods.

All concentrations are reported for the flavonoids as aglycones. Using the hydrolysis procedure, only aglycones appeared in the chromatogram. For analysis of direct extracts of the foods, both aglycones and glycosylated compounds were present for the flavanones. Peaks for naringenin (aglycone), narirutin (naringenin-7-*O*-rutinoside), naringin (naringenin-7-*O*-neohesperoside), hesperetin (aglycone), and hesperidin (hesperetin-7-*O*-rutinoside) were quantified using calibration standards, and the final results are reported as total naringenin and hesperetin.

**Comparison to Flavonoid Database Values.** The results from this study were compared to two Special Interest Databases released by the Nutrient Data Laboratory (4): (1) the flavonoid database (FDB) and (2) the proanthocyanidin database (PDB). The latter database was established on the basis of different subsamples of the same regional food samples analyzed in this study.

Data in the FDB were compiled by the Nutrient Data Laboratory from a literature survey in 2003 and updated in 2005. Data from the FDB (4) are listed in **Tables 2–4** in the rows labeled FDB. Data from this study are listed in the rows labeled FCL. When possible, the FDB values and the results from the current study (FCL) are compared using a *t* test (shaded cells). Differences that were significant at the 95% confidence limit have been highlighted by a black border. In general, there are no observable patterns for the cases of significant differences in the data. Neither data set (FDB or FCL) was consistently higher or lower than the other. For the flavan-3-ols, all but one of the significant differences occurs for the catechins and epicatechins, and mainly for the fruit group. This is not surprising because catechins and epicatechins are the main flavan-3-ols in fruits and few data have been reported for the vegetables and nuts. However, there are some points that deserve discussion.

Differences in the reported values can arise from a number of sources: nonrepresentative sampling, different cultivars, different growing and processing conditions, and analytical bias.

In **Tables 2–4**, a number of significant differences occur as a result of nonrepresentative sampling; that is, a comparison is based on a single value ( $n = 1$ ) in the FDB. Five such cases can be seen for the flavan-3-ols in apples and cranberries in **Table 2A**. Other instances can be observed for vegetables (**Table 3A**) and nuts (**Table 4A**). In these cases, the *t* test is based on the assumption that the standard deviation obtained for this study is valid for both measurements. However, characterization of the concentration of a flavonoid in a food by a single sample is not statistically valid, especially if the variance is large (see Sample Variation). Consequently, a comparison based on a single measurement is problematic.

Significant differences arise from the analysis of different cultivars. Kurilich et al. (15) analyzed 50 varieties of broccoli and determined that the levels of vitamins A, C, and E can vary by an order of magnitude. Cultivar sources are well documented in the FDB. In many cases, international cultivars, many unavailable in the United States, have been incorporated into the FDB to provide as comprehensive a listing as possible. Conversely, the cultivars analyzed in this study are unknown. The national sampling protocol designed by the Nutrient Data Laboratory was a market survey that called for the purchase of foods at retail outlets without regard to botanical variety. In some cases, specific cultivars were sampled when they are expected to be recognized by the average consumer, for example, varieties of apples, lettuce, and potatoes. However, most consumers are unaware, for example, of the many varieties of almonds, bananas, blackberries, blueberries, broccoli, cranberries, and strawberries. For this study, whichever cultivar was in the store was purchased with no documentation.

The FDB and FCL values for catechins and epicatechins in blueberries (**Table 2A**), although noticeably different, are not statistically significant because of the large standard deviations (RSDs of almost 200%) associated with the FDB values. The FDB values for both flavan-3-ols are based on 12 different high-bush and low-bush varieties. The catechin and epicatechin values ranged from 0 to 129 and from 10 to 246 mg/100 g, respectively. Catechin and epicatechin values in nectarines and peaches are each based on five different cultivars and have RSDs of 50–85%. The FDB values for catechins in bananas are based on a single study that analyzed varieties from Tenerife in the Canary Islands. Information regarding cultivar is not listed in the database. However, the database does provide the journal reference from which the data were obtained. Thus, anyone can access the information.

Significant differences can be seen for delphinidin in blueberries, for cyanidin and pelargonidin in cherries, and for pelargonidin in strawberries (**Table 2B**). In these cases, the FDB values are based on data for Canadian and Spanish cultivars. Significant differences are seen for myricetin and quercetin in blackberries, blueberries, cranberries, strawberries, and onions (**Table 2C**). In each case, the RSDs are high (50–150%) and a variety of cultivars were used. Of the seven cranberry cultivars, two came from The Netherlands and Finland.

All four of the detectable flavonoids in almonds (catechin, epicatechin, naringenin, and quercetin), were lower in this study than for the FDB values. The FDB values are based on eight cultivars collected in California. RSDs were approximately 50%. For the present study, loose almonds (not canned, bagged, or in jars) were collected in stores. The source and variety of the almonds were not known but were considered representative of the U.S. food supply.



Table 2. (Continued)

**(B) Anthocyanins (Continued)**

Description	NNDB No	Source	Cya			Del			Mal			Pelar			Peon			Pet		
			n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s
Artichokes, ocean mist, boiled	99362	FCL FDB	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
Avocados, raw	09037	FCL FDB	6	0.3	0.3	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0
Bananas, raw	09040	FCL FDB	8	0.0	0.0	8	7.4	3.3	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
Blackberries, raw	09042	FCL FDB	4	81.0	30.7	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0
Blueberries, raw	09050	FCL FDB	7	16.9	9.6	7	48.1	13.1	7	54.6	12.0	7	0.0	0.0	7	7.8	1.8	7	29.6	7.7
Cherries, sweet, raw	09070	FCL FDB	4	31.0	6.3	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	4.0	1.4	4	0.0	0.0
Cranberries, raw	09078	FCL FDB	4	39.8	4.5	4	9.5	1.0	4	0.0	0.0	4	0.0	0.0	4	36.8	6.0	4	0.0	0.0
Dates, raw	09087	FCL FDB	6	1.7	1.6	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0
Figs, raw	09089	FCL FDB	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
Grapefruit, pink & red, raw	09112	FCL FDB	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0
Kiwi fruit, fresh, raw	09148	FCL FDB	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
Kiwi, gold, raw	97079	FCL FDB	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
Melons, cantaloupe, raw	09181	FCL FDB	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0
Melons, honeydew, raw	09184	FCL FDB	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0
Nectarines, raw	09191	FCL FDB	8	2.2	1.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
Peaches, raw	09236	FCL FDB	7	1.4	1.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0
Pears, green cultivars, w peel, raw	97075	FCL FDB	8	12.2	7.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
Pineapple, all varieties, raw	09266	FCL FDB	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
Pineapple, extra sweet, raw	09430	FCL FDB	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0
Plums, raw	09279	FCL FDB	8	12.5	11.5	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
Plums, black diamond, w peel, raw	97077	FCL FDB	2	37.6	20.1	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0
Plums, dried (prunes), uncooked	09291	FCL FDB	7	0.3	0.9	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0
Raisins, seedless	09298	FCL FDB	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0	5	0.0	0.0
Raspberries, raw	09302	FCL FDB	6	25.0	2.6	6	0.0	0.0	6	0.0	0.0	6	1.0	2.3	6	0.0	0.0	6	0.0	0.0
Strawberries, raw	09316	FCL FDB	7	2.5	1.3	7	0.0	0.0	7	0.0	0.0	7	5.4	9.3	7	0.0	0.0	7	0.0	0.0
Watermelon, raw	9326	FCL FDB	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0

(C) Flavanones, Flavones, and Flavonols

Description	NNDB No	Source	Flavanones						Flavones						Flavonols								
			Hesp			Nari			Api			Luteo			Kaem			Myr			Quer		
			n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s
Apples, fuji, raw	97066	FCL FDB	4	0	0	4	0	0	4	0.0	0.0	2	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	2.1	2.0
Apples, gala, w peel, raw	97067	FCL FDB	3	0.0	0.0	3	0.0	0.0	4	0.0	0.0	2	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	3.6	1.3
Apples, gold del, wo peel, raw	97068	FCL FDB	2	0.0	0.0	1	0.0	0.0	2	0.0	0.0	1	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.6	0.0
Apples, gold del, w peel, raw	97069	FCL FDB	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0				4	0.0	0.0	4	0.0	0.0	4	3.5	0.7
Apples, granny smith, w peel, raw	97070	FCL FDB	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	2	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	3.5	0.7
Apples, red del, wo peel, raw	97071	FCL FDB	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.3	0.5
Apples, red del, w peel, raw	97072	FCL FDB	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	2	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	3.8	3.6

Table 2. (Continued)

Description	NNDB No	Source	(C) Flavanones, Flavones, and Flavonols (Continued)														
			Flavanones			Flavones			Flavonols								
			Hesp	Nari	Api	Luteo	Kaem	Myr	Quer								
n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s			
Artichokes, ocean mist, boiled	99362	FCL	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
		FDB															
Avocados, raw	09037	FCL	7	0.0	0.0	7	0.0	0.0	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0
		FDB							1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
Bananas, raw	09040	FCL	7	0.0	0.0	7	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0
		FDB							1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
Blackberries, raw	09042	FCL	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	2	0.0	0.0	4	0.0	0.0
		FDB							1	0.0	0.0	1	0.0	0.0	12	0.1	0.0
Blueberries, raw	09050	FCL	8	0.0	0.0	8	0.0	0.0	7	0.0	0.0	3	0.0	0.0	7	0.0	0.0
		FDB							1	0.0	0.0	1	0.8	0.0	24	1.8	2.4
Cherries, sweet, raw	09070	FCL	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0
		FDB							2	0.0	0.0	2	0.0	0.0	2	0.0	0.0
Cranberries, raw	09078	FCL	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0				4	16.6	1.8
		FDB													14	0.1	0.0
Dates, raw	09087	FCL	5	0.0	0.0	5	0.0	0.0	6	0.0	0.0	3	0.0	0.0	6	0.0	0.0
		FDB													6	0.0	0.0
Figs, raw	09089	FCL	5	0.0	0.0	5	0.0	0.0	8	0.0	0.0	8	0.0	7.0	8	0.0	0.0
		FDB													8	0.0	0.0
Grapefruit, pink & red, raw	09112	FCL	7	0.0	0.0	6	26.5	6.4	7	0.0	0.0	4	0.0	0.0	7	0.0	0.0
		FDB	1	1.2	0.0	1	45.0	0	1	0.0	0.0	1	1.4	0.0	3	0.0	0.0
Kiwi fruit, fresh, raw	09148	FCL	5	0.0	0.0	5	0.0	0.0	2	0.0	0.0	1	0.0	0.0	2	0.0	0.0
		FDB							1	0.0	0.0	1	2.2	0.0	1	0.0	0.0
Kiwi, gold, raw	97079	FCL	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
		FDB															
Melons, cantaloupe, raw	09181	FCL	7	0.0	0.0	7	0.0	0.0	3	0.0	0.0	2	0.0	0.0	3	0.0	0.0
		FDB							2	0.0	0.0	2	1.3	1.8	2	0.0	0.0
Melons, honeydew, raw	09184	FCL	5	0.0	0.0	5	0.0	0.0	2	0.0	0.0	1	0.0	0.0	2	0.0	0.0
		FDB															
Nectarines, raw	09191	FCL	7	0.0	0.0	7	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
		FDB															
Peaches, raw	09236	FCL	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	4	0.0	0.0	7	0.0	0.0
		FDB							2	0.0	0.0				2	0.0	0.0
Pears, green cultivars, w peel, raw	97075	FCL	6	0.0	0.0	6	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0
		FDB													4	0.0	0.0
Pineapple, all varieties, raw	09266	FCL	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
		FDB							1	0.0	0.0	1	0.0	0.0	1	0.0	0.0
Pineapple, extra sweet, raw	09430	FCL	5	0.0	0.0	5	0.0	0.0	3	0.0	0.0	2	0.0	0.0	3	0.0	0.0
		FDB															
Plums, raw	09279	FCL	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0
		FDB							2	0.0	0.0	2	0.0	0.0	2	0.0	0.0
Plums, black diamond, w peel, raw	97077	FCL	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	1	0.0	0.0	2	0.0	0.0
		FDB													2	0.0	0.0
Plums, dried (prunes), uncooked	09291	FCL	3	0.0	0.0	3	0.0	0.0	7	0.0	0.0	4	0.0	0.0	7	0.0	0.0
		FDB							1	0.0	0.0	1	0.0	0.0	2	0.0	0.0
Raisins, seedless	09298	FCL	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0	2	0.0	0.0	5	0.0	0.0
		FDB							1	0.0	0.0	1	0.0	0.0	2	0.0	0.0
Raspberries, raw	09302	FCL	3	0	0	3	0	0	6	0.0	0.0	2	0.0	0.0	6	0.0	0.0
		FDB							1	0.0	0.0	1	0.0	0.0	9	0.1	0.0
Strawberries, raw	09316	FCL	6	0.0	0.0	7	0.0	0.0	7	0.0	0.0	3	0.0	0.0	7	0.0	0.0
		FDB				1	1.8	0	3	0.0	0.0	3	0.0	0.0	69	0.5	0.1
Watermelon, raw	9326	FCL	7	0.0	0.0	7	0.0	0.0	3	0.0	0.0	1	0.0	0.0	3	0.0	0.0
		FDB							2	0.0	0.0	2	0.9	1.3	2	0.0	0.0

<sup>a</sup> Gray shading indicates where *t* tests can be made. Black borders indicates where values are significantly different,  $P < 0.05$  with a *t* test. Abbreviations: C, catechin; CG, catechin gallate; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GC, gallic acid; GCG, gallic acid gallate; Cya, cyanidin; Del, delphinidin; Mal, malvidin; Pelar, pelargonidin; Peon, peonidin; Pet, petunidin; Hesp, hesperetin; Nari, naringenin; Api, apigenin; Luteo, luteolin; Kaem, kaempferol; Myr, myricetin; Quer, quercetin. FCL, results from Food Composition Lab, this study; FDB, results listed in the flavonoid database.

Growing and processing conditions can also influence the concentration of flavonoids in foods. Flavonoids are frequently classified as environmental compounds because they are often produced in direct response to environmental conditions. It has been documented that flavonoid content is dependent on ultraviolet light and CO<sub>2</sub> levels (16, 17). With these sources of variation, it is not surprising that there are differences in the flavonoid concentrations for similar foods collected from different regions at different times. This study was a market

study and was not designed to permit deconvolution of cultivar and growing and processing variability.

The most common sources of analytical bias are calibration accuracy, extraction efficiency, and correct identification of chromatographic peaks. Calibration accuracy is usually checked using a standard reference material (SRM) issued by the National Institute for Standards and Technology (and similar international organizations). Results for catechin and epicatechin were verified using SRM 2384, baking chocolate. There are no





Table 3. (Continued)

(B) Anthocyanins (Continued)																				
Description	NNDB No	Source	Cya			Del			Mal			Pelar			Peon			Pet		
			n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s
Potatoes, red, w skin, baked	11358	FCL FDB	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
Radishes, raw	11429	FCL FDB	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	25.0	10.3	7	0.0	0.0	7	0.0	0.0
Tomatoes, red, ripe, raw	11529	FCL FDB	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0
Tomatoes, red, ripe, cooked	11530	FCL FDB	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.0	0.0

(C) Flavanones, Flavones, and Flavonols

Description	NNDB No	Source	Flavanones						Flavones						Flavonols								
			Hesp		Nari		Api		Luteo		Kaem		Myr		Quer								
			n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s			
Broccoli, raw	11090	FCL	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.4	0.4			
		FDB							4	0.0	0.0	5	0.8	1.8	17	4.0	5.6	4	0.0	0.0	7	4.0	4.5
Broccoli, cooked, boiled, drained	11091	FCL	1	0.0	0.0	1	0.0	0.0	4	0.0	0.0	1	0.0	0.0	4	0.0	0.0	4	0.0	0.0	4	0.0	0.0
		FDB													1	1.4	0.0				1	1.1	0.0
Broccoli, raab, raw	11096	FCL	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	2.3	3.2			
		FDB																					
Broccoli, raab, cooked	11097	FCL	4	0.0	0.0	4	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	1.1	1.8
		FDB																					
Celery, raw	11143	FCL	5	0.0	0.0	5	0.0	0.0	7	1.3	0.7	4	0.0	0.0	8	0.0	0.0				8	0.0	0.0
		FDB							8	4.6	4.8	8	1.3	1.7							1	3.5	0.0
Carrots, baby, raw	11960	FCL	4	0.0	0.0	4	0.0	0.0	2	0.0	0.0	1	0.0	0.0	2	0.0	0.0	2	0.0	0.0	2	0.0	0.0
		FDB										0.0	0.0					0.0				0.1	
Lettuce, butterhead, raw	11250	FCL	4	0.0	0.0	4	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	3.9	4.7
		FDB													3	0.0	0.0				7	1.4	0.9
Lettuce, green leaf, raw	11253	FCL	2	0.0	0.0	2	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	7.8	4.4
		FDB							6	0.4	0.9	11	0.5	0.7	10	0.0	0.0	2	0.5	0.6	21	4.7	6.2
Lettuce, iceberg, raw	11252	FCL	4	0.0	0.0	4	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.1	0.1
		FDB							5	0.5	1.2	6	0.1	0.2	19	0.2	0.0	5	0.2	0.5	15	1.6	2.4
Lettuce, red leaf, raw	11257	FCL	5	0.0	0.0	5	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	8.3	2.6
		FDB							2	0.0	0.0	3	4.2	4.1	2	0.0	0.0				4	22.5	21.9
Lettuce, cos or romaine, raw	11251	FCL	6	0.0	0.0	6	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	5.8	2.1
		FDB										1	0.0	0.0							1	1.0	0.0
Onions, sweet, raw	11294	FCL	5	0.0	0.0	5	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	7.6	5.0
		FDB							2	0.0	0.0	2	0.0	0.0	11	1.0	0.8	7	2.4	1.7	11	16.4	15.1
Potatoes, russet, w skin, raw	11353	FCL	4	0.0	0.0	4	0.0	0.0	3	0.0	0.0	1	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	1.7	1.7
		FDB																					
Potatoes, white, w skin, raw	11354	FCL	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	2	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.5	0.5
		FDB																					
Potatoes, red, w skin, raw	11355	FCL	2	0.0	0.0	2	0.0	0.0	3	0.0	0.0	2	0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.7	0.6
		FDB																					
Potatoes, russet, w skin, baked	11356	FCL	6	0.0	0.0	6	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.7	0.6
		FDB																					
Potatoes, white, w skin, baked	11357	FCL	6	0.0	0.0	6	0.0	0.0	6	0.0	0.0	3	0.0	0.0	6	0.0	0.0	6	0.0	0.0	6	1.2	1.1
		FDB																					
Potatoes, red, w skin, baked	11358	FCL	7	0.0	0.0	7	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	1.4	0.6
		FDB																					
Radishes, raw	11429	FCL	3	0.0	0.0	3	0.0	0.0	7	0.0	0.0	3	0.0	0.0	7	0.0	0.0	7	0.0	0.0	7	0.0	0.0
		FDB							3	0.0	0.0	3	0.0	0.0	7	0.9	0.3	3	0.0	0.0	4	0.0	0.0
Tomatoes, red, ripe, raw	11529	FCL	6	0.0	0.0	6	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.5	0.3
		FDB				1	1.5	0.0	4	0.0	0.0	6	0.0	0.0	46	0.1	0.0	5	0.2	0.4	37	0.6	0.7
Tomatoes, red, ripe, cooked	11530	FCL	5	0.0	0.0	5	0.0	0.0	8	0.0	0.0	4	0.0	0.0	8	0.0	0.0	8	0.0	0.0	8	0.8	0.8
		FDB							1	0.0	0.0	1	0.0	0.0	2	0.0	0.0	1	0.0	0.0	1	0.5	0.0

<sup>a</sup> Gray shading indicates where *t* tests can be made. Black borders indicate where values are significantly different,  $P < 0.05$  with a *t* test. Abbreviations: C, catechin; CG, catechin gallate; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GC, gallic acid; GCG, gallic acid gallate; Cya, cyanidin; Del, delphinidin; Mal, malvidin; Pelar, pelargonidin; Peon, peonidin; Pet, petunidin; Hesp, hesperetin; Nari, naringenin; Api, apigenin; Luteo, luteolin; Kaem, kaempferol; Myr, myricetin; Quer, quercetin; FCL, results from Food Composition Lab, this study; FDB, results listed in the flavonoid database.

certified values for any other flavonoid compounds. The extraction process used in this study is well documented (11) and has been shown to be 95% efficient for the flavonoids determined in this study. Identification of peaks is based on retention time and use of the spectral matching routine (for UV-vis spectra from 200 to 600 nm) available as a part of the HPLC software. Analysis of pure standards or samples with standard additions is a simple method of checking peak identities in cases of doubt.

One concern about the hydrolysis method was the possibility of a high bias for cyanidin, delphinidin, and pelargonidin resulting from the hydrolysis of proanthocyanidins found in some foods. In general, the levels of these three anthocyanidins were similar between this study and the FDB (Tables 2B and 3B) for those foods reported to be high in proanthocyanidins (7). However, cyanidin values for all apples tended to be somewhat higher for this study compared to FDB values, although the absolute concentrations were low (0.8–8.1 mg/100 g).



**Table 5.** Comparison of Total Flavan-3-ol Monomers in Fruits and Nuts (Milligrams per 100 g of Fresh Weight)<sup>a</sup>

food material	ref 5, total monomers <sup>b</sup>	this study, sum of C and EC <sup>c</sup>
Fruits		
apples, Fuji	6.5 ± 1.7	5.8 ± 5.5 (4)
apples, Gala	5.9 ± 0.4	3.2 ± 1.5 (3)
apples, Golden Delicious, with peel	4.7 ± 0.2	3.6 ± 2.2 (4)
apples, Granny Smith	7.5 ± 1.0	4.9 ± 1.7 (4)
apples, Red Delicious, with peel	9.6 ± 0.9	7.6 ± 6.4 (4)
apples, Red Delicious, without peel	6.8 ± 0.9	5.1 ± 0.2 (2)
avocados	1.0 ± 0.8	0.4 ± 0.5 (7)
blackberries	3.7 ± 2.2	1.0 ± 1.4 (4)
blueberries	3.4 ± 0.5	2.8 ± 1.3 (8)
cherries	4.2 ± 1.1	5.7 ± 2.9 (4)
cranberries	7.3 ± 1.5	5.3 ± 1.3 (4)
dates	ND	ND (5)
kiwis	0.6 ± 0.5	0.1 ± 0.2 (5)
nectarines	1.9 ± 1.2	0.6 ± 0.8 (7)
peaches	4.7 ± 1.4	3.4 ± 0.8 (7)
pears	2.7 ± 1.5	9.5 ± 6.5 (6)
plums	11.3 ± 3.4	6.2 ± 4.2 (8)
raspberries	4.4 ± 3.4	5.6 ± 4.5 (3)
strawberries	4.2 ± 0.7	3.2 ± 1.8 (6)
Nuts		
almonds	7.8 ± 0.9	0.4 ± 0.2 (4)
cashews	6.7 ± 2.9	0.9 ± 0.5 (6)
hazelnuts	9.8 ± 1.6	1.4 ± 1.1 (5)
pecans	17.2 ± 2.5	8.0 ± 1.4 (7)
pistachios	10.9 ± 4.3	4.4 ± 3.0 (7)
walnuts	6.9 ± 3.4	ND (4)

<sup>a</sup>  $n = 4-8$ . <sup>b</sup> Total monomer values in ref 5 represent catechin and epicatechin.

<sup>c</sup> Sum of catechin and epicatechin for foods in **Tables 2A, 3A, and 4A**.

Delphinidin values for blueberries were also significantly higher from this study even though blueberry proanthocyanidins produce only cyanidin (6). We concluded from these observations that these differences were due to natural variation of the flavonoid content of foods.

Data from the PDB (4) are compared to the flavan-3-ol data from this study in **Table 5**. Duplicate subsamples of the regional samples collected for this study were analyzed for oligomeric and polymeric flavan-3-ols by scientists at the Arkansas Children's Nutrition Center (5). As part of these analyses, values for monomeric flavan-3-ols (primarily catechins and epicatechins) were also generated (5). These data were provided to USDA to establish the PDB (4). **Table 5** compares the total monomers (catechin and epicatechin) from the PDB to the sums of catechin and epicatechin (**Tables 2A and 4A**) measured in this study. For the fruits, the values from this study are generally lower than those in the PDB. Pears and raspberries are notable exceptions, for which the values from this study are higher. For the nuts, the values from this study are consistently lower than those in the PDB. It should be remembered that the values in **Table 5** were obtained using different methods and that the samples had been frozen for 2 years ( $-80\text{ }^{\circ}\text{C}$  as freeze-dried powders) before they were made available to Arkansas Children's Nutrition Center (7).

**Seasonal Variation.** Samples were collected twice during the year (two passes) because of the seasonal nature of fruits, vegetables, and nuts. Whereas consumers can find most produce available the whole year, the sources of the produce and, most likely, the cultivars are different. Thus, it was anticipated that seasonal variations might result in differences in flavonoid levels for the two passes; however, the only food that displayed a seasonal variation was blueberries (**Table 6**). It can be seen that statistically significant differences were found for cyanidin, delphinidin, malvidin, peonidin, petunidin, and quercetin but

**Table 6.** Seasonal Variation of Blueberries (Milligrams per 100 g of Fresh Weight)

flavonoid	pass 1	pass 2	probability pass 1 = pass 2
cyanidin	10.0 ± 2.4 (4)	26.0 ± 6.8 (3)	0.006 <sup>a</sup>
delphinidin	39.2 ± 5.5 (4)	60.1 ± 9.6 (3)	0.014 <sup>a</sup>
malvidin	46.8 ± 2.3 (4)	65.1 ± 11.6 (3)	0.025 <sup>a</sup>
peonidin	6.8 ± 0.9 (4)	9.4 ± 1.6 (3)	0.038 <sup>a</sup>
petunidin	23.4 ± 1.1 (4)	1.7 ± 0.3 (3)	0.000 <sup>a</sup>
quercetin	11.0 ± 1.7 (4)	4.4 ± 4.4 (3)	0.036 <sup>a</sup>
epicatechin	1.4 ± 1.1 (3)	0.3 ± 0.3 (4)	0.111
epigallocatechin	1.2 ± 0.6 (3)	1.5 ± 0.5 (4)	0.530
catechin	2.8 ± 1.3 (3)	1.8 ± 0.6 (4)	0.216

<sup>a</sup>  $P < 0.05$ .

**Table 7.** Variability of Flavonoids in the Food Supply

flavonoid	average of standard deviation for each food (%)			
	fruits	vegetables	nuts	total
flavan-3-ols <sup>a</sup>				
C	87 (17) <sup>b</sup>		77 (4)	87 (21)
CG	146 (12)			146 (12)
EC	104 (18) <sup>b</sup>		79 (5)	99 (23)
ECG	233 (3)		167 (1)	217 (4)
EGC	88 (15)		73 (4)	85 (19)
EGCG	133 (16)	225 (1)	74 (2)	132 (19)
GC	250 (1)			250 (1)
GCG	100 (12)		102 (4)	100 (16)
anthocyanidins				
cyanidin	71 (20)	191 (1)	51 (4)	73 (25)
delphinidin	28 (3)		34 (1)	30 (4)
malvidin	22 (1)			22 (1)
pelargonidin	204 (2)			204 (2)
peonidin	25 (3)			25 (3)
petunidin	27 (1)			27 (1)
flavanones				
hesperetin				
naringenin	24 (1)			24 (1)
flavones				
apigenin		57 (1)		57 (1)
luteolin				
flavonols				
myricetin	11 (1)			11 (1)
quercetin	84 (23)	92 (1)	123 (1)	86 (25)
regional samples	98 (149)	128 (4)	79 (26)	97 (179)
individual samples				168 (537) <sup>c</sup>

<sup>a</sup> Abbreviations are the same as for **Table 1**. <sup>b</sup> Average RSD (number of foods).

The number of measurements within each food ranged from 4 to 8. <sup>c</sup> Each food sample analyzed was a composite of three samples collected from different locations;  $n_{\text{individual}} = 3 \times n_{\text{composite}}$  and  $\text{RSD}_{\text{individual}} = \text{sqrt}(3) \times \text{RSD}_{\text{composite}}$ .

not for catechin, epicatechin, and epigallocatechin. In all other cases, the mean values were sufficiently similar or the variation between regions was sufficiently high to make the differences statistically insignificant.

**Sample Variation.** As mentioned earlier, flavonoid content is known to be highly dependent on the cultivar and growing and processing conditions. Consequently, the variation in concentration for a systematic sampling of foods is equally as interesting as the concentration levels. **Table 7** presents the average standard deviation associated with the determination of each flavonoid in each food. For example, for the analysis of epicatechin (EC) in fruit, there were 18 fruits for which the RSD was nonzero. The average RSD for the 18 fruits was 104%. The number of regional samples analyzed in each food to produce these RSDs can be found in **Tables 2-4**. In all, there were 179 nonzero RSDs for the flavonoids in **Tables 2-4**, and the average RSD was 97%. Each regional sample was a

**Table 8.** Blueberry Quality Assurance Analyses<sup>a</sup>

flavonoid	n	average	standard deviation	RSD
cyanidin	20	20.5	2.9	14%
delphinidin	20	36.3	4.0	11%
malvidin	20	36.5	6.9	19%
peonidin	20	9.1	0.8	9%
petunidin	20	25.4	3.6	14%
quercetin	20	14.5	2.2	15%

<sup>a</sup> Analyses were performed between October 1, 2000, and May 8, 2003.

composite of samples from three locations, so the theoretical RSD for individual samples is 168%.

**Table 8** presents the RSDs for the determination of six flavonoids in a blueberry in-house control material. This material was analyzed periodically for 2.5 years during the course of the project. The RSDs for the six flavonoids ranged from 9 to 19%. These RSDs are higher than expected for a well-controlled analytical method but can be explained by considering the method of analysis. The hydrolysis method uses the analytical results from 10 aliquots to extrapolate to the flavonoid concentration at time zero using pseudo-first-order kinetics. Thus, the RSD will minimally be  $\sqrt{10}$  times greater than that for a method based on a single determination. Extrapolation beyond the time range of the measured values further increases the RSD. Thus, the RSDs for the hydrolysis method are larger than desired, but they are still 5–10 times less than the composited food RSD and 8–17 times less than that for individual foods.

The large average RSD shown in **Table 7** most likely arises from differences in cultivars and growing conditions. These factors cannot be identified in this study because samples were purchased off the shelf in the manner the average consumer would purchase them. The high RSDs suggest that it is difficult to make an a priori prediction as to the flavonoid content of a food item one is about to consume. As has been succinctly stated, the food you eat is not the food you analyzed. The body, however, will act as an integrator. The level of exposure to flavonoids in foods eaten over an extended period of time can be predicted by the values in this study.

**Summary.** This study characterizes the concentration and variation of flavonoids in the U.S. food supply. The results are based on analytical determinations for more than 60 foods collected across the United States using a statistically valid sampling protocol. In general, values from this study agree well with available national and international data in the existing USDA database. Considerable variation was found between foods and within foods. The mean values reported in this study are inclusive of varietal and seasonal variations and will be useful for studying the health benefits of flavonoid intake.

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Received for review May 25, 2006. Revised manuscript received October 19, 2006. Accepted October 30, 2006.

JF061478A