

Abstract

Evidence to suggest an association of dietary flavonoids and reduction in cancer risk is consistent (Neuhouser, 2004). Flavonoids have also been associated with reduction in the risk of cardiovascular diseases (Huxley and Neil 2003). Reliable databases to estimate dietary flavonoid intakes are thus essential to study the health benefits and reduction in risks of chronic diseases. Nutrient Data Laboratory (NDL) of the U.S. Department of Agriculture (USDA) has developed databases for 3 different groups of flavonoids: monomeric flavonoids, polymeric proanthocyanidins and isoflavones. A database for monomeric flavonoids for 26 selected compounds in five subclasses (flavonols, flavones, flavanones, flavans and anthocyanidins) was released in March 2003. The database contained values for 225 selected foods. Most of the data came from the analytical studies conducted in the countries other than the United States. Therefore the NDL procured nationally representative samples of 59 fruits, vegetables and nuts through the USDA's National Food and Nutrient Analysis Program (NFNAP). It was also observed that analysts frequently concentrated on quantifying one or two particular subclasses of flavonoids for the lack of a suitable analytical method to separate and quantify all the five subclasses simultaneously. A kinetics method was developed by Merken, et al (2001) Food Composition Laboratory (FCL) to separate and quantify 26 compounds representing all five subclasses. Food samples collected by the NDL through NFNAP were analyzed by FCL of the USDA using this method. The most significant finding from national sampling was the high degree of variability; an average RSD of 116% for composited samples or 197% for individual samples. These data will be incorporated into the revised database and released, on NDL's website (www.nal.usda.gov/fnic/foodcomp). In addition, literature data published since 2002 were also collected and evaluated. Acceptable analytical data from approximately 90 studies have been or will be combined with the previous data. The new database will provide data on flavonols, flavones, flavanones, flavans and anthocyanidins for more than 400 selected foods and will be more representative of flavonoids content of U.S. foods and subsequent dietary intakes.

Introduction

Evidence to suggest an association of dietary flavonoids and reduction in cancer risk from four cohort and six case-control studies is consistent (Neuhouser 2004). Protection against cancer may be achieved through antioxidant activity of flavonoids. Antioxidant mechanisms include free radical chain breaking, metal chelating, and singlet oxygen quenching (Yao, et al 2004). Flavonoids have also been associated with reduction in the risk of cardiovascular diseases (Huxley and Neil 2003) through several possible effects which include reducing inflammation and endothelial damage by scavenging nitric oxide radicals (NO) (van Acker, et al 1995) and inhibiting inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzyme expression (Raso, et al 2001).

There are more than 5000 flavonoid compounds; but dietary flavonoids consist mainly of five subclasses of monomeric flavonoids (flavonols, flavones, flavanones, flavans and anthocyanidins), polymeric proanthocyanidins and isoflavones. Nutrient Data Laboratory (NDL) has prepared three separate databases for these three groups of flavonoids. The database for monomeric flavonoids, USDA Database for the Flavonoid Content of Selected Foods, was released on NDL's web site (www.nal.usda.gov/fnic/foodcomp) in March 2003. It contained values for 225 selected foods for 26 compounds representing the five subclasses. Acceptable analytical data from 97 articles were included in this database after reviewing 450 articles published since 1970. The database revealed many gaps in the knowledge of flavonoids composition (Table 1). As a result, NDL and the Food Composition Lab (FCL), as part of the National Food and Nutrient Analysis Program, initiated the updating of the 2003 flavonoid database. This program had the following objectives:

- To analyze nationally representative U.S. samples of 59 fruits, vegetables and nuts for 26 flavonoid compounds from five subclasses of monomeric flavonoids using a method developed by Merken, et al (2001).
- To collect and evaluate analytical flavonoids data from published literature from 2002 to 2004.
- To update the USDA Flavonoids Database by compiling all acceptable analytical data (analyzed and published).

Table 1. Gaps in the knowledge of flavonoids composition for the 2003 database

- Very limited U.S. data
- Values from single studies for many foods
- Lack of data on anthocyanidins
- Many analytical studies for a single class of compounds only
- Lack of analytical method to separate and quantify all major flavonoids from all the classes simultaneously
- Lack of Standard Reference Materials for flavonoids

Methods

Sampling plan and sample handling:

In cooperation with the Produce for Better Health Foundation, a program was developed for the analysis of 59 fruits, nuts and vegetables. Samples were collected in two different seasons from retail outlets in 12 cities around the United States and shipped to the Food Analysis Laboratory Control Center (FALCC) at Virginia Polytechnic Institute and State University in Blacksburg, Virginia for preparation and compositing according to protocols developed by FALCC and NDL. Tomatoes, potatoes, broccoli, and broccoli raab were also cooked. For all produce, any inedible portions were removed and weighed. After preparation, four regional composite samples were created for each food. Uncooked samples were then freeze-dried to maintain stability. Aliquots of each composite were then sent to FCL for analysis of flavonoids.

Analysis:

Samples were analyzed for 26 compounds from the five subclasses (Table 2) using the method developed by Merken, et al (2001) (Figure 1).

Literature Search:

Several databases (CAB abstracts, Biosis Previews, Agricola, Food science and Technology Abstracts) were searched using key words for flavonoids and relevant published articles were retrieved from 2002 to 2004. Articles containing analytical data on 26 selected compounds in edible foods were separated.

Data Management:

- Values for glucosides were converted into aglycone forms using molecular weights
- All the values were reported as mg/100g of fresh weight of edible part (dry wt. values were converted to fresh wt. using given or standard moisture content).
- Values for beverages were adjusted to 100g weight using specific gravities.
- Trace values were quantified as 0.71x LOQ (Limit of Quantitation) if the LOQ was available.
- A zero value reported in the database is a true zero (below the Limit of Detection - LOD). Therefore a missing value does not necessarily imply a zero value, but an unavailable value.

Data evaluation and compilation:

Data were evaluated using the data quality evaluation system developed by the USDA (Holden, et al 2002) for 5 categories of information: sampling plan, sample handling, number of samples, analytical method and analytical quality control and ratings were assigned on the scale of 0 to 20. The sum of the ratings for the 5 categories is used to generate a Quality Index (QI) and a Confidence Code (CC), an indicator of the data quality.

Analytical data generated by the USDA and acceptable literature data from 2002 to 2004 were aggregated with values in the earlier database to create the updated database. The database contains the mean value (mg/100g), standard error of means (SEM), minimum (Min.), and maximum (Max.) for each flavonoid compound for each food. The database also contains a confidence code (CC), providing an indication of data reliability to the user. In addition, the list of references from where the data were used for each mean value is provided. The database will be released on the NDL's web site: www.nal.usda.gov/fnic/foodcomp.

Table 2. Flavonoid subclasses in the Database

1. Flavonols: Quercetin, Kaempferol, Myricetin, Isorhamnetin
2. Flavan-3-ols: Catechins, Epicatechins, Epicatechin3-gallate, Epigallocatechin, Epigallocatechin 3-gallate, Galloocatechin, Theaflavin, Thraflavin 3-3-digallate, Theaflavin 3-gallate, Theaflavin 3-gallate, Thearubigins
3. Flavones: Apigenin, Luteolin
4. Flavanones: Hesperetin, Naringenin, Eriodictyol
5. Anthocyanidins: Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin

Figure 1. Method of Analysis

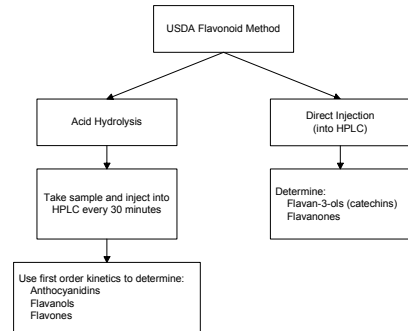


Table 3. Average %RSD of Measured Flavonoids

Flavonoid	Foods	Average %RSD
Quercetin	42	98
EC	29	115
C	29	111
EGC	27	115
Cyanidin	27	84
EGCG	25	159
GCG	20	132
CG	15	139
Average		116

Table 4. Comparison of values for selected foods and flavonoids

Flavonoid	USDA Update (2005) mg/100g ± SE (N)	USDA Database (2003) mg/100g ± SE (N)
Broccoli, raw		
Kaempferol	1.6 ± 0.58 (8)	6.2 ± 0.02 (9)
Quercetin	1.8 ± 1.50 (9)	3.2 ± 0.06 (9)
Luteolin	1.0 ± 0.33 (12)	0.0 ± 0.00 (21)
Beans, snap, green, raw		
Kaempferol	0.10 (1)	0.4 ± 0.01 (40)
Luteolin	0.0 (1)	0.0 (12)
Quercetin	3.4 ± 0.92 (6)	2.7 ± 0.03 (44)
Lettuce, green leaf		
Quercetin	6.2 ± 1.01 (34)	2.0 ± 0.43 (5)
Apigenin	0.2 ± 0.18 (13)	0.0 (1)
Luteolin	0.5 ± 0.11 (24)	0.0 (1)
Myricetin	0.1 ± 0.10 (9)	0.0 (1)
Oranges, raw		
Hesperetin	26.1 ± 3.54 (19)	32.7 ± 1.58
Naringenin	16.0 ± 2.83	11.2 ± 0.14
Cherries, sweet, raw		
Cyanidin	31.0 ± 3.17 (4)	111.4 ± 3.71 ** 1
Pelargonidin	0.0 (4)	0.8 ± 0.16 (4) **
Peonidin	4.0 ± 0.72 (4)	5.2 ± 0.23 (7)

¹PR > F .0001, SAS 8.02 (Cary, NC) using NPAR1WAY procedure with Wilcoxon scores and Exact test.

Table 5. New foods with anthocyanidin values (mg/100 g) (blanks denote values not available)

	Cyanidin	Delphinidin	Malvidin	Pelargonidin	Peonidin	Petunidin
Fruits						
Bananas	0.0	7.4	0.0	0.0	0.0	0.0
Bilberries	112.6	161.9	54.4		51	51
Blackberries	85.7	0.0	0.0	0.0	0.0	0.0
Blueberries, frozen	4.4	21.6	49.6	0.0	0.5	18.2
Chokeberries	535.8			1.4		
Cranberries	39.8	9.5	16.6	36.9	0.0	0.0
Currants, black	85.6	181.1	1.2	0.7	3.9	
Grapes, red	0.4	2.6	48.6	0.0	1.6	2.3
Plums, black	22.0	0.0	0.0	0.0	0.0	0.0
Strawberries, raw	2.0	0.3	0.0	33.0	0.0	0.0
Strawberries, frozen	1.3	0.0		19.3		
Vegetables						
Cabbage, red, raw	8.2	0.1		0.0		
Cowpeas (Blackeyed peas), mature seeds	94.7	94.6	34.3		11.1	27.8
Eggplant, raw	0.0	0.0		0.0		
Radish	0.0	0.0	0.0	25.0	0.0	0.0
Beverages						
Black currant nectar	16.0	27.8				
Grape Juice	0.6	0.5		0.0		

Results

Analysis of nationally representative samples of fruits, vegetables and nuts: The new mean values generated by the analysis of samples from U.S. foods compared well with data collected from the literature. Values in the 2003 database came mostly from international sources and differences in the cultivars and growing conditions may have influenced the large variation in the values. This was confirmed by the high degree of variability observed in the analysis of U.S. samples—an average RSD of 116% for samples composited by regions and RSD of 197% for individual samples (Table 3). High variability was observed in the database as well. In most cases there was no significant difference between data collected for the 2003 database vs. the 2005 database (Table 4). Also, there was no significant difference observed between samples analyzed in the U.S. vs. those analyzed in other countries for the 2005 database.

Literature Search: Approximately 255 articles were retrieved through literature searches on various databases. Out of these only 94 articles contained acceptable analytical data. Reasons for rejecting the remaining 161 articles for inclusion in the database are as follows: 16 Review articles; 40 Health-related articles; 35 Analytical method development studies including identification or characterization only; 11 No analytical data (consumption patterns, studies to investigate antioxidant or other properties of flavonoids); 59 Non-useable data (unacceptable methods, values for total flavonoids or compounds other than the selected 26 compounds, values in ranges or on dry wt. Basis, values for non-edible foods, etc).

The updated database will contain approximately 400 selected foods compared to 225 in the earlier version.

- About 20 new vegetables, 15 new fruits, 10 nuts, 2 new herbs added (raw, cooked, frozen, frozen cooked, canned, fruit juices etc.)
- Anthocyanidin values for 12 fruits, 5 vegetables and 2 beverages for which values were added, where previously missing (Table 5).
- Apple varieties are reported separately (e.g. red Delicious, golden Delicious, Gala, Granny Smith)
- Data for specific lettuce varieties (Red Leaf, Romaine, Butterhead)
- Values for selected foods and compounds compare fairly well between the 2005 and 2003 databases, except for kaempferol in raw broccoli, quercetin in green leaf lettuce and cyanidin in sweet cherries (Table 4)

References:

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