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Determination of Flavonoids and Phenolics and Their Distribution in Almonds

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Limited information is available concerning the qualitative and quantitative composition of polyphenolic compounds, especially flavonoids, in almonds. We determined total phenols, flavonoids, and phenolic acids in California almond (*Prunus dulcis*) skins and kernels among the principal almond varieties (Butte, Carmel, Fritz, Mission, Monterey, Nonpareil, Padre, and Price) with high-performance liquid chromatography (HPLC)/electrochemical detection and UV detection. Liquid chromatography/tandem mass spectrometry under identical HPLC conditions was utilized to verify identities of the predominant flavonoids and phenolic acids. Total phenols ranged from 127 (Fritz) to 241 (Padre) mg gallic acid equivalents/100 g of fresh weight. The analyses were compiled to produce a data set of 18 flavonoids and three phenolic acids. The predominant flavonoids were isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside (in combination), catechin, kaempferol-3-*O*-rutinoside, epicatechin, quercetin-3-*O*-galactoside, and isorhamnetin-3-*O*-galactoside at 16.81, 1.93, 1.17, 0.85, 0.83, and 0.50 mg/100 g of fresh weight almonds, respectively. Using the existing approach of calculating only the aglycone form of flavonoids for use in the U.S. Department of Agriculture nutrient database, whole almonds would provide the most prevalent aglycones of isorhamnetin at 11.70 (3.32), kaempferol at 0.60 (0.17), catechin at 1.93 (0.55), quercetin at 0.72 (0.20), and epicatechin at 0.85 (0.24) mg/100 g of fresh weight (mg/oz serving), respectively. These data can lead to a better understanding of the mechanisms of action underlying the relationship between almond consumption and health-related outcomes and provide values for whole and blanched almonds suitable for inclusion in nutrient databases.

KEYWORDS: Almonds; flavonoids; phenols; HPLC; electrochemical array detection; LC/MS/MS

INTRODUCTION

Fruits and nuts contain a wide variety of phenolic acids and flavonoids that are predominantly conjugated with sugars or other polyols via *O*-glycosidic bonds or ester bonds (*1*). **Figure 1** shows the basic structures and substitution patterns for the major compounds discussed in this paper. The composition and distribution of these conjugated compounds substantially underly the differences observed in the culinary, physiological, and medicinal properties of these plant foods. However, the absence of detailed characterizations of these constituents markedly limits our understanding of their biological properties, especially those that appear to confer health benefits (*2*−*4*). For example, observational studies reveal an inverse correlation between nut consumption and risk of coronary heart disease (*5*−*7*). While some of the bioactivity of tree nuts, e.g., improved lipid profiles (*8*−*11*), protection against low-density lipoprotein (LDL) oxidation (*12, 13*), and chemoprevention (*14*), is presumed to be due to their content of arginine, fiber, mono- and polyunsaturated fat, vitamin E, and other nutrients, the presence of phenolic acids and polyphenols may also contribute to their health profile (*15*−*18*). While the U.S. Department of Agriculture has recently created nutrient databases of flavonoids (*19*), including separate files on proanthocyanidins (*20*) and isoflavones (*21*), they remain incomplete and present more data describing these constituents as their aglycone rather than their natural glycoside forms. We report here a characterization of the identity, quantity, and distribution of the flavonoids and phenolic acids in the context of the total phenol content of the eight principal almond varieties. These data can help lead to a better understanding of the mechanisms of action underlying the relationship between almond consumption and health-related outcomes in experimental and human studies.

MATERIALS AND METHODS

Chemicals and Reagents. The following analytical grade reagents were obtained from Sigma Co. (St. Louis, MO): sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, and Folin−Ciocalteu's phenol reagent. All organic solvents and glacial acetic acid were purchased from Fisher Co. (Fair Lawn, NJ). Standard materials

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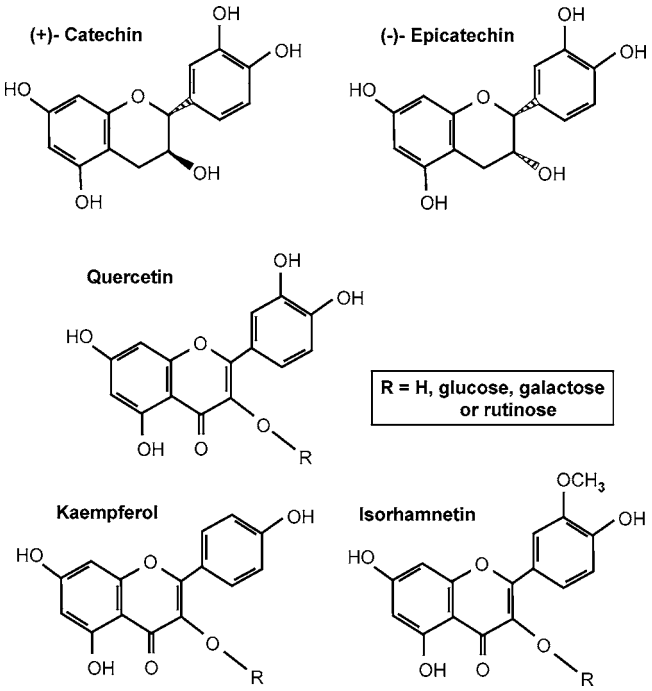

Figure 1. Structures of the major almond flavonoids. Sugars, when conjugated at the three position on the C ring, may be glucose, galactose, or rutinose, a disaccharide comprised of rhamnose (6-deoxy-L-mannose) and glucose.

for quercetin, catechin, (−)-epicatechin, kaempferol, isorhamnetin, protocatechuic acid, and *p*-hydroxybenzoic acid were obtained from Sigma Co. Dihydroxykaempferol was obtained from Apin Chemicals Ltd. (Abingdon, United Kingdom). Vanillic acid and eriodictyol were obtained from Indofine Chemical Co., Inc. (Belle Mead, NJ). All other standards were procured from Extrasynthase (Genay, France).

Sample Acquisition, Extraction, and Purification. Kernels from the eight most commonly grown California almonds species (*Prunus dulcis*) (out of 32 varieties), i.e., Butte, Carmel, Fritz, Mission, Monterey, Nonpareil, Padre, and Price, were provided in raw, whole form by the Almond Board of California. The varieties were collected from across a wide variety of orchards throughout the state and pooled so that no individual orchard was disproportionately represented. The collection process occurred during 2004 and 2005. The almonds were stored in the dark at 4 °C until they were separated into individual aliquots for extraction and analysis. In an industrial setting, water blanching is employed to remove the skin (seed coat), a process that has the potential to extract substantial amounts of flavonoids. To determine the total flavonoid and phenolic acid content and distribution in the almonds, we analyzed skins, blanch water, and kernels (blanched almonds) separately. Briefly, 100 g of whole almonds was blanched in 175 mL of boiling distilled water for 2.5 min, a procedure designed to mimic the typical industrial blanching process, and the skins were removed by hand. The volume of blanch water produced from 100 g of almonds was recorded and used to calculate the flavonoid content extracted from the skin. After a cleanup step via centrifugation and filtration through a Millex-HV 0.45 μm filter (Millipore, Billerica, MA), the total phenol content in the samples was determined by the Folin−Ciocalteu reaction. Individual flavonoids were analyzed by high-performance liquid chromatography (HPLC)/electrochemical detection (ECD) using a 16 channel Coulometric Array Detector System (ESA, Inc., Chelmsford, MA.).

Almond kernels and skins were lyophilized for 7 days, and their weights were recorded. Prior to flavonoid extraction, kernels and skins were pulverized under liquid nitrogen with a mortar and pestle. One gram of pulverized kernels was extracted two times with 10 mL of hexane (1:10, w/v) to remove lipids, and after it was centrifuged at 1000*g*, the pellet was dried under nitrogen. The pellet and the almond skins were extracted at 1:15 (w/v) with HCl:H_2O:methanol (3.7:46.3:50, v/v/v) by a two-step, sequential extraction over 16 h at 4 °C. The methanolic extract was retained and evaporated under nitrogen. The residue was reconstituted in 10 mM phosphate buffer for the Folin−Ciocalteu assay or the initial aqueous mobile phase for HPLC analysis.

Total Phenols. Total phenols were assayed colorimetrically with the Folin−Ciocalteu method (*22*) as modified by Singleton et al. (*23*). Briefly, 2.5 mL of Folin−Ciocalteu reagent (diluted 10-fold), 2 mL of 7.5% sodium carbonate, and 0.5 mL of phenolic extract were mixed well. After the reaction mixture was heated at 45 °C for 15 min, absorbance was measured at 765 nm on a UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Ten millimolar phosphate buffer was employed as a blank. The total phenols content was expressed as μmol/L gallic acid equivalents (GAE) and then converted to mg GAE/100 g of almond component or whole almond as fresh weight.

HPLC/ECD Analysis of Almond Flavonoids. The extraction residue was dissolved in the aqueous mobile phase defined below, and the phenolic acids and polyphenols were characterized by HPLC/ECD using the Coularray instrument (ESA, Inc.), which includes model 582 pumps, a 542 autosampler, and a 5600A Coularray detector. Separation was achieved using a 250 mm × 4.6 mm i.d., 4 μm Synergy RP-Max column (Phenomenex, Torrance, CA) maintained at 32 °C, using a gradient from 30 to 99% methanol in 1% aqueous formic acid over 120 min and a flow rate of 0.2 mL/min. To run identical chromatography conditions on the HPLC/ECD and liquid chromatograpy (LC)/tandem mass spectrometry (MS/MS) systems, sodium acetate (salts are required for ECD and contraindicated for MS) was introduced into the Coularray system after chromatographic separation by use of a third HPLC pump set to deliver 0.2 mL/min of aqueous buffer (150 mmol/L citric acid and 50 mmol/L ammonium acetate). Detection was achieved using an array of 13 coulometric electrochemical detectors with potentials applied in 60 mV increments from 60 to 720 mV. The effluent of the Coularray was directed to a SPD-10AV UV/vis detector (Shimadzu) connected to deliver its readout signal to the Coularray system and software. The chromatography was monitored at 280 and 365 nm for quantification and to ensure comparable chromatography between the HPLC/ECD and LC/MS/MS systems. The quantity of individual almond flavonoids was calculated according to concentration curves constructed with authentic standards. The intra-assay coefficient of variation (CV) for flavonoids determined by HPLC ranged from 1.24 to 5.17% across all of the flavonoids determined. The interassay CV ranged from 2.23 to 10.92%. Six flavonoids were used for CV calculations: catechin, epicatechin, eriodictyol, isorhamnetin, naringenin, and quercetin.

LC/MS/MS Identification of Almond Flavonoids. LC/MS/MS was used in addition to authentic standards to verify the identities of the flavonoids in almonds. Compounds were separated on an Agilent 1100 HPLC (Palo Alto, CA) fitted with a 250 mm × 4.6 mm id., 4 μm Synergy RP-Max column (Phenomenex) at 32 °C using the gradient described above from 30 to 99% methanol in 1% aqueous formic acid over 120 min and a flow rate of 0.2 mL/min. The column eluent was directed through an Agilent UV G1315A diode array detector (PDA) monitoring from 250 to 700 nm into a Bruker Esquire ion trap MS/MS fitted with an electrospray interface operating in negative ion mode with alternating MS and MS/MS scans from *m*/*z* 150 to 1000. MS/MS scans of unknown compounds in the almonds were compared with authentic flavonoid standards. All metabolites were identified based on a complete match of their HPLC retention time (rt), UV absorption (250, 280, and 365 nm), *m*/*z* of their molecular ions, and MS/MS fragmentation patterns.

RESULTS AND DISCUSSION

Flavonoids and related polyphenolics are ubiquitous in plants and contribute to the sensory qualities of foods and beverages, particularly astringency and bitterness (*24*, *25*). These phytochemicals are generally classified as dietary antioxidants because the hydroxyl moieties within their conjugated π-electron system readily donate hydrogen to reactive oxygen and nitrogen species in vitro (*26*). However, the low bioavailability of these compounds in the presence of much higher in vivo concentra-

Table 1. Total Phenolic Content in Skins, Blanch Water, Kernels, and Whole Almonds

	mg GAE/100 g fresh weight				
variety	skins	blanch water	kernels	whole almonds	total phenols from skin %
Butte	17.1	116.5	64.4	198.0	67.4
Carmel	12.9	53.8	66.5	133.2	50.1
Fritz	9.9	50.3	66.6	126.8	47.5
Mission	18.0	70.8	70.6	159.4	55.7
Monterey	19.6	56.2	66.9	142.7	53.2
Nonpareil	26.4	99.2	67.7	193.3	65.0
Padre	21.2	153.9	65.7	240.8	72.7
Price	26.8	101.8	70.9	199.5	64.4

tions of antioxidants such as ascorbate, glutathione, and α-tocopherol and their potent bioactivity in pathways for signal transduction and gene expression suggests that their putative health benefits are mediated substantially via these latter mechanisms (*27*, *28*).

The composition of flavonoids in plants is influenced by both genetic factors, e.g., variety, and environmental conditions, including exposure to fungi and bacteria, pests, weather, and UV light. Furthermore, relative ripeness, processing, and storage can also significantly influence the flavonoid content of food plants (*29*, *30*). Variety, geographical origin, and cultivation methods also have been shown to affect the lipid, mineral, and vitamin content of tree nuts (*31*, *32*). Thus, for this study, almond samples were collected over a 2 year period and from multiple California orchards growing the same varieties. Samples were pooled and mixed such that no variety from an individual orchard was disproportionately represented in the study.

Tree nuts are high in fat and low in water content with a dry weight ~96% and a fat dry weight of 50−60% (*33*, *34*). Consistent with the approach of other laboratories, we removed lipids from the almond kernels with a hexane extraction and characterized compounds remaining in the dry matter. Investigation potential varietal differences in lipid content would be of interest but is beyond the scope of this study. Lipids were removed from the kernel to prevent potential interference in subsequent phenolics and chromatographic methods. We blanched the almonds to determine the distribution of phenolic compounds between the skins and the kernel. The total phenolic content of almonds in skin, blanch water, and kernels expressed as mg GAE/100 g fresh weight of whole almonds is listed in **Table 1**. The standard deviations of all samples did not exceed 5% of the mean. Extraction and analysis of total phenolics from almond skins typically provided standard deviations of 2−3% of means. The interassay CV for the Folin−Ciocalteu assay was 3.3%. The value for whole almonds was calculated by summing the value of individual components, including blanch water. While the total phenolic content for most tree nuts has not been reported, Anderson et al. (*35*) found walnuts to contain 1604 mg GAE/100 g shelled nuts. Their screening of walnuts with LC/MS using evaporative light scattering detection revealed ellagic acid and ellagitannins (valoneic acid dilactone, pedunculagin). While quercetin pentoside was also identified in walnuts, their results indicate that walnut polyphenolics are principally from nonflavonoid classes, although identification of all of the phenolic components remains to be established. Wu et al. (*36*) have reported a similar total phenolics value of 1556 mg GAE/100 g for lyophilized walnuts and 418 mg GAE/100 g for almonds using an accelerated solvent extraction method with acetone:H_2O:acetic acid (70:29.5:0.5; v/v/v). Using a slight modification of the extraction method of Anderson et al. (*35*), Kornsteiner et al. (*34*) determined total phenols at 239 mg GAE/100 g in chopped almonds extracted with acetone:H_2O (75:25; v/v) containing 526 μmol/L sodium metabisulfite. Our extraction method, using sequential extractions over 16 h at 4 °C with HCl:H_2O:methanol (3.7:46.3:50; v/v/v), is similar to the method used by Ryan et al. (*37*) who recovered total phenols from *Olea europaea*. While different extraction methods may account, in part, for the difference in reported total phenols among investigators for the various nuts, our results for almonds are similar to those reported by Kornsteiner et al. (*34*) who found the distribution of almond phenolics to be predominantly within the "seed skin" (~80%) with only 47 mg GAE/100 g in the kernel. We found a mean 60% of almond phenolics were present in the skin. Differences in total phenolics between almond varieties were primarily due to differences in the content of skin (e.g., 60.2 and 128.6 mg GAE/100 g in Fritz and Price, respectively), while the content in the kernels was similar between varieties, within the range of 64.4−70.9 mg GAE/100 g.

Table 2. Almond Flavonoids and Phenolic Acids Identified by LC/MS/MS and HPLC/ECD

peak	compound	mol. ion $[M-H]^-$	primary fragment (MS/MS)	rt (min)	UV λ (nm)	EC potential (mV)
1	catechin	289	245, 205	21.3	270	660
2	procatechuic acid	153	109	24.3	250	600
3	epicatechin	289	245, 205	27.6	260	480
4	*p*-hydroxy-benzoic acid[a]	137		35.4	250	600
5	vanillic acid	167		37.8	250	720
6	quercetin-3-*O*-galactoside	463	301	46.3	360	420
7	naringenin-7-*O*-glucoside	433	271	51.2	280	720
8	quercetin-3-*O*-rutinoside	609	300	51.5	360	360
9	quercetin-3-*O*- glucoside	463	301	53.2	360	420
10	dihydroxykaempferol	287	259	55.7	280	540
11	kaempferol-3-*O*-galactoside[a]	447	285	56.7	360	480
12	isorhamnetin-3-*O*-galactoside	477	315	58.4	360	600
13	kaempferol-3-*O*-glucoside[a]	447	285	59.7	340	480
14	kaempferol-3-*O*-rutinoside[a]	593	285	60.5	360	600
15	isorhamnetin-3-*O*-rutinoside (with isorhamnetin-3-*O*-glucoside)	623 (477)	315, 300 (315, 300)	61.5 (62.5)	360 (360)	660 (660)
16	eriodictyol	287	151	66.7	280	360
17	quercetin	301	179, 151	74.9	365	300
18	naringenin[a]	271	177, 151	78.7	280	720
19	kaempferol	285		87.6	365	300
20	isorhamnetin	315	300	89.8	365	240

[a] Quantified using UV.

The identities and detection characteristics (rt, electrochemical, UV, and mass data) of the flavonoids and phenolics identified in almonds by chromatography are listed in **Table 2**. The compound numbers in **Table 2** correspond to the peak numbers in **Figure 2A**,**B**. The close match in rts for almond flavonoid analysis between LC/MS with electrospray ionization and HPLC/ECD is shown in a typical set of chromatographs (**Figure 2A**,**B**), respectively. The close match of rts permitted the use of LC/MS/MS, in conjunction with authentic standards and the use of a PDA, as a cross-platform verification of the identities of the compounds being quantified by HPLC/ECD or UV detection. While the majority of compounds yielded good oxidation signals as indicated by the response levels for HPLC/ECD (**Figure 2B**), five compounds, *p*-hydroxy-benzoic acid, kaempferol-3-*O*-galactoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, and naringenin (shown in the inset in **Figure 2B**), displayed relatively low HPLC/ECD responses and a better response with UV detection; therefore, these compounds were quantified using UV detection. These chromatography conditions were not capable of separating isorhamnetin glucoside and isorhamnetin rutinoside by rt or oxidation potential, so the

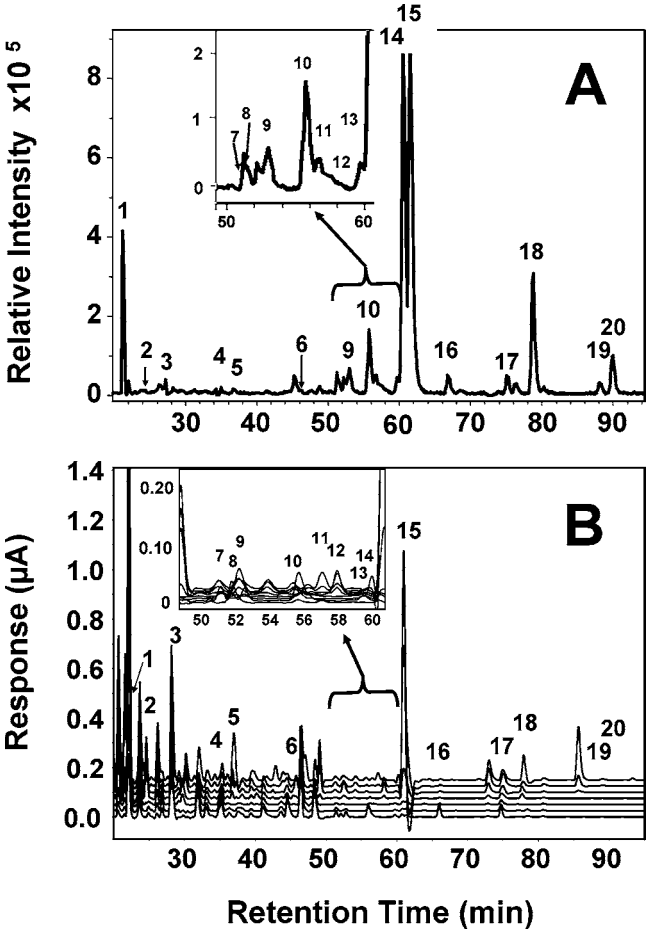

Figure 2. Typical chromatograms of almond skin extract from (**A**) LC/MS/MS and (**B**) HPLC/ECD. Peak numbers correspond to compounds listed in **Table 2**. Five compounds, *p*-hydroxy-benzoic acid (4), kaempferol-3-*O*-galactoside (11), kaempferol-3-*O*-glucoside (13), kaempferol-3-*O*-rutinoside (14), and naringenin (18), showed relatively low electrochemical responses (see inset box) and so were analyzed by UV photodiode array detection.

reported value represents the coelution of both compounds. Nine compounds were extracted that could not be identified with LC/MS/MS. However, four of these compounds produced patterns suggesting their identity as dihydroxy-kaempferol glycoside (rt, 24.4 min; *m*/*z* 449 → 287 → 259), eriodictyol galactoside (rt, 41.5 min; *m*/*z* 449 → 287 → 151), eriodictyol glucoside (rt, 57.9 min; *m*/*z* 449 → 287 → 151), and stigmasterol (rt, 34.7 min; *m*/*z* 457 → 411), a lipophilic plant sterol that has previously been reported in almonds at ~11 mg/100 g (*38*). Five of these compounds did not yield fragmentation patterns.

Distribution data combined from the analysis of individual flavonoids in all eight varieties are shown in **Table 3**. Interestingly, as so little of the total almond weight is skin (4.43 ± 0.30%) and that eight of the 19 flavonoids and three phenolic acids determined were found exclusively in the skin, on average, 94 ± 7.9% of individual flavonoids originated from the skin (**Table 3**). This differential in flavonoid distribution also indicates that the majority of compounds contributing to the total phenolic content of the kernel are not flavonoids. This observation is consistent with the role of flavonoids as phytoalexins, which are localized to the skin layer surrounding seeds and nuts protecting them against bacterial, fungal, and other environmental stresses (*39*). It is worth noting that with our blanching conditions designed to mimic commercial processing, neither kaempferol nor quercetin was extracted into the water.

Table 3. Percent Distribution of Identified Flavonoids and Phenolic Acids in Skins, Blanch Water, and Kernels from Almonds[a]

	%			
compound	skins	blanch water	kernels	compounds from skin
catechin	35.7 ± 7.4	55.6 ± 11.8	8.8 ± 6.2	91.2 ± 6.2
procatechuic acid	17.6 ± 1.9	70.2 ± 4.6	12.2 ± 3.3	87.8 ± 3.3
epicatechin	33.9 ± 2.2	62.1 ± 3.8	4.0 ± 2.9	96.0 ± 2.9
p-hydroxy-benzoic acid	17.5 ± 6.3	82.5 ± 6.3	ND	100
vanillic acid	18.2 ± 6.8	72.6 ± 10.4	9.3 ± 5.1	90.7 ± 5.1
quercetin-3-*O*-galactoside	41.4 ± 13.6	58.6 ± 13.6	ND	100
naringenin-7-O-glucoside	16.1 ± 9.0	73.4 ± 4.7	10.5 ± 7.9	89.5 ± 7.9
quercetin-3-*O*-rutinoside	43.7 ± 19.7	56.3 ± 19.7	ND	100
quercetin-3-*O*-glucoside	24.5 ± 11.4	75.5 ± 11.4	ND	100
dihydroxykaempferol	50.8 ± 18.2	49.2 ± 18.2	ND	100
kaempferol-3-*O*-galactoside	36.4 ± 12.5	43.5 ± 20.9	20.1 ± 29.2	79.9 ± 29.2
isorhamnetin-3-*O*-galactoside	35.0 ± 7.0	61.7 ± 8.4	3.2 ± 2.8	96.8 ± 2.8
kaempferol-3-*O*-glucoside	39.0 ± 7.0	44.5 ± 4.3	16.6 ± 9.2	83.4 ± 9.2
kaempferol-3-*O*-rutinoside	40.4 ± 6.7	52.6 ± 8.5	7.0 ± 3.0	93.0 ± 3.0
isorhamnetin-3-*O*-rutinoside (and isorhamnetin-3-*O*-glucoside)	28.5 ± 7.6	68.6 ± 9.0	2.9 ± 2.3	97.1 ± 2.3
eriodictyol	48.7 ± 6.8	51.3 ± 6.8	ND	100
quercetin	100	ND	ND	100
naringenin	34.1 ± 6.2	37.8 ± 11.4	28.1 ± 11.5	71.9 ± 11.5
kaempferol	100	ND	ND	100
isorhamnetin	47.2 ± 8.4	50.6 ± 12.3	2.1 ± 6.1	97.9 ± 6.1

[a] ND, not detected.

The summary results of the flavonoids and phenolic acids in whole almonds of the eight varieties studied are shown in **Table 4**. Standard deviations are not presented for space and clarity purposes; however, they are typically less than 5%.

Using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, Frison-Norrie and Sporns (*40*, *41*) identified four flavonol glycosides, isorhamnetin glucoside, isorhamnetin rutinoside, kaempferol glucoside, and kaempferol rutinoside, in skins from 16 almond varieties with concentrations comparable to those observed in this study. Contrasts in the data may result from the limited ionizability of some flavonoids with MALDI-TOF MS. In addition, MALDI-TOF MS cannot differentiate isorhamnetin-3-galactoside from isorhamnetin-3-glucoside as they have identical molecular weights. Recently, Wijeratne et al. (*42*) demonstrated the antioxidant efficacy of almond ethanolic extracts and, using HPLC rts and spectroscopic analysis, identified but did not quantify protocatechuic acid with quercetin, isorhamnetin, morin, quercetin 3-*O*-rhamnoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, and isorhamnetin 3-*O*-glucoside as the major flavonoids in almond kernels, skins, and hulls. Using high-resolution one- and two-dimensional nuclear magnetic resonance and atmospheric pressure chemical ionization quadrupole MS, Sang et al. (*43*, *44*) identified, but did not quantify, 11 phenolic compounds from almond skins after extraction with ethyl acetate and *n*-butanol: 3′-*O*-methylquercetin 3-*O*-*β*-D-glucopyranoside, 3′-*O*-methylquercetin 3-*O*-*β*-D-galactopyranoside, 3′-*O*-methylquercetin 3-*O*-*R*-L-rhamnopyranosyl-(1-6)-*β*-D-glucopyranoside, kaempferol 3-*O*-α-L-rhamnopyranosyl-(1-6)-*β*-D-glucopyranoside, naringenin 7-*O*-*β*-D-glucopyranoside, catechin, protocatechuic acid, vanillic acid, *p*-hydroxybenzoic acid, 3-prenyl-4-*O*-*β*-D-glucopyranosyloxy-4-hydroxylbenzoic acid, and ursolic acid. Using HPLC with UV diode array detection, Takeoka and Dao (*45*) quantified three isomers of chlorogenic acid in Nonpareil almond hulls, with 5-*O*-caffeoylquinic acid (chlorogenic acid), 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), and 3-*O*-caffeoylquinic acid (neochlorogenic acid) in a ratio of 80:15:5 and a total concentration of 42.52 ± 4.50 mg/100 g fresh weight.

This detailed analysis of the phenolic and polyphenolic content of almonds as well as of other tree nuts can lead to a

Table 4. Quantities of Flavonoids and Phenolic Acids in Eight Almond Varieties

	μg/100 g							
compound	Butte	Carmel	Fritz	Mission	Monterey	Nonpareil	Padre	Price
catechin	1771.5	1358.0	950.5	1631.5	1139.6	2382.3	1860.2	3856.8
procatechuic acid	335.8	321.6	445.0	132.1	212.9	157.4	274.4	249.9
epicatechin	748.7	322.9	545.3	658.3	499.9	1213.4	653.6	1272.6
p-OH-benzoic acid	2.7	5.6	3.6	3.1	6.5	3.6	3.0	4.7
vanillic acid	147.4	201.4	95.7	130.7	111.8	297.0	145.2	246.8
quercetin-3-*O*-galactoside	765.0	610.6	315.9	877.1	241.1	1029.2	1218.6	1258.3
naringenin-7-*O*-glucoside	185.7	374.8	154.1	176.9	110.9	88.9	159.2	87.8
quercetin-3-*O*-rutinoside	284.5	373.1	59.9	529.6	193.4	101.0	231.1	299.0
quercetin-3-*O*-glucoside	65.0	145.9	41.6	157.7	116.5	46.4	43.1	133.5
dihydroxykaempferol	39.3	39.8	78.6	185.6	302.0	117.1	55.3	77.7
kaempferol-3-*O*-galactoside	4.9	9.6	4.6	35.1	19.8	20.5	4.6	5.6
isorhamnetin-3-*O*-galactoside	573.6	690.5	716.5	579.6	924.8	297.6	494.0	429.6
kaempferol-3-*O*-glucoside	15.4	18.7	23.5	12.7	14.3	41.5	7.9	23.2
kaempferol-3-*O*-rutinoside	1011.5	1432.2	963.0	877.2	739.2	1297.0	708.0	1227.8
isorhamnetin-3-*O*-rutinoside and -glucoside	18764.7	16374.9	9859.2	12558.5	11061.5	19074.4	13514.7	15891.4
eriodictyol	83.9	303.9	94.6	573.6	230.9	393.0	28.6	331.6
quercetin	29.6	21.9	23.0	26.7	26.0	26.6	25.4	35.2
naringenin	45.5	104.7	67.9	180.2	109.1	123.0	18.5	120.7
kaempferol	0.7	3.0	1.6	2.8	3.5	17.3	0.0	3.8
isorhamnetin	86.2	120.4	118.3	94.1	106.1	462.1	87.1	112.1
total flavonoids	24961.6	23133.5	14562.4	19423.1	16169.8	27189.3	19532.7	25671.0

better understanding of the mechanisms of action underlying the relationship between their consumption and health-related outcomes such as a reduction in serum cholesterol (*46*) and inflammation (*47*) and increased resistance of LDL to oxidation determined in experimental and clinical protocols. In addition, this information can also contribute directly to existing flavonoid databases for use in observational studies investigating diet−health relationships. However, in this regard, limitations may exist, as products containing almonds do not identify variety on their label and few consumers know about the differential use of almond varieties in baked items, confectionery, dairy, and prepared foods. As the predominant flavonoid across all of the varieties is isorhamnetin (as the 3-*O*-rutinoside or 3-*O*-glucoside), representing about 70% of the total, major errors in estimated intake are unlikely. Nonetheless, an integrated approach to including almond flavonoids in nutrient databases might be considered as California grows over 850 million pounds of almonds annually, supplying over 85% of the world supply. In 2005, the eight varieties reported here represented 89.83% of the total sales of 32 varieties produced in California with the percent of total receipts from U.S. Department of Agriculture inspections as follows: Nonpareil, 35.75%; Carmel, 15.36%; Butte, 12.27%; Monterey, 6.61%; Padre, 4.68%; Fritz, 4.32%; Mission, 2.87%; Price, 2.86%; and where Butte/Padre were combined and sold together, 5.11%.

Data on the flavonoid content of almond varieties can be combined with information on market sales to provide a basis for averaging nutrient composition values (**Table 5**). For example, using the existing approach by the U.S. Department of Agriculture and others of calculating only the aglycone form of flavonoids for use in nutrient databases, whole almonds would provide the most prevalent aglycones of isorhamnetin at 11.70 (3.32), kaempferol at 0.60 (0.17), catechin at 1.93 (0.55), quercetin at 0.72 (0.20), and epicatechin at 0.85 (0.24) as mg/100 g (mg/serving), respectively. This approach allows for comparisons of flavonoid intake from different foods, e.g., on a weight basis, showing that almonds provide similar amount of flavonols as red onions, but 9-fold more isorhamnetin than white onions (*48*, *49*). The kaempferol and quercetin contents of almonds are comparable to that of broccoli and its concentration of catechin is between that of brewed black and green tea (*19*). Interestingly, in vitro studies indicate the greatest degree of cytoprotection by flavonoids against cell death induced by oxidative stress was found by aglycones of myricetin and quercetin and glycosides of catechin, epicatechin, and kaempferol, the principal flavonoids in almonds (*50*). Developing hypotheses about and testing whole foods such as almonds (or their component parts such as kernels and skins) require a detailed understanding of their nutrient and phytochemical composition. Thus, further studies of the composition of tree nuts such as almonds, including potential changes in content affected by postharvest processing and cooking, are warranted.

Table 5. Quantities of Almond Flavonoids and Phenolic Acids Adjusted for Individual Varietal Contribution to the Food Supply[a]

compound	μg/100 g	μg/oz[b]
catechin	1933.7	548.2
procatechuic acid	240.7	68.2
epicatechin	847.2	240.1
p-hydroxy-benzoic acid	4.0	1.1
vanillic acid	217.6	61.6
quercetin-3-*O*-galactoside	830.2	235.3
naringenin-7-O-glucoside	166.6	47.2
quercetin-3-*O*-rutinoside	210.4	59.6
quercetin-3-*O*-glucoside	78.9	22.4
dihydroxykaempferol	101.9	28.9
kaempferol-3-*O*-galactoside	14.5	4.1
isorhamnetin-3-*O*-galactoside	504.0	142.9
kaempferol-3-*O*-glucoside	27.1	7.7
kaempferol-3-*O*-rutinoside	1170.5	331.8
isorhamnetin-3-*O*-rutinoside and -glucoside	16809.7	4765.5
eriodictyol	288.1	81.7
quercetin	26.2	7.4
naringenin	100.7	28.5
kaempferol	8.5	2.4
isorhamnetin	255.4	72.4
total flavonoids	23890.4	6772.92

[a] On the basis of total receipts from U.S. Department of Agriculture inspections from August 1, 2004 through February 28, 2005 (see Results and Discussion). [b] One serving was defined as 1 oz by the Food and Drug Administration.

NOTE ADDED AFTER ASAP PUBLICATION

The original posting of June 2, 2006, has been corrected. Some quantities of almond aglycones in the abstract and the last paragraph of the Results and Discussion section have been corrected in the revised ASAP posting of June 9, 2006.

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