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Simultaneous Determination of All Polyphenols in Vegetables, Fruits, and Teas

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Polyphenols, which have beneficial effects on health and occur ubiquitously in plant foods, are extremely diverse. We developed a method for simultaneously determining all the polyphenols in foodstuffs, using HPLC and a photodiode array to construct a library comprising retention times, spectra of aglycons, and respective calibration curves for 100 standard chemicals. The food was homogenized in liquid nitrogen, lyophilized, extracted with 90% methanol, and subjected to HPLC without hydrolysis. The recovery was 68−92%, and the variation in reproducibility ranged between 1 and 9%. The HPLC eluted polyphenols with good resolution within 95 min in the following order: simple polyphenols, catechins, anthocyanins, glycosides of flavones, flavonols, isoflavones and flavanones, their aglycons, anthraquinones, chalcones, and theaflavins. All the polyphenols in 63 vegetables, fruits, and teas were then examined in terms of content and class. The present method offers accuracy by avoiding the decomposition of polyphenols during hydrolysis, the ability to determine aglycons separately from glycosides, and information on simple polyphenol levels simultaneously.

KEYWORDS: Polyphenol determination; flavonoids; anthocyanins; catechins; vegetables; fruits; teas

INTRODUCTION

Polyphenols in vegetables, fruits, and teas can prevent degenerative diseases including cancers through antioxidative action and/or the modulation of several protein functions. For example, the intake of antioxidative polyphenols reduces coronary heart disease mortality (*1*) by suppressing the oxidation of low-density lipoprotein (*2*). Polyphenols exhibit agonism and/or antagonism of carcinogenesis-related receptors such as epidermal growth factor (*3*), arylhydrocarbon receptor (*4*), and estrogen receptor β (*5*). They modulate the secretion of cytokines, regulating the cell cycle (*6*−*9*) and expression of protein kinases in tumor cell proliferation (*10*, *11*). They induce the expression of anticarcinogenic enzymes (*12*) or inhibit induction of cancer-promoting enzymes (*13*−*15*). In animal experiments, an oral dose of polyphenols suppressed the carcinogenesis of several carcinogens (*16*−*20*). Polyphenols also show actions for vasorelaxation (*21*, *22*) and antiallergy (*23*).

It is important to determine the amounts and species of polyphenols in vegetables, fruits, and teas. The number of natural polyphenols has been estimated to be over one million, because they generally occur as glycosides, and the sugar species and binding forms show great variety (*24*, *25*). However, the bioactivity is attributed to aglycon structures, not to sugar moieties. The antioxidative potency is due mainly to the orthodiol (catechol) structure in aglycons (*26*, *27*). The specificity of interaction with proteins depends on the steric structures of respective aglycons, with the sugar moieties disrupting the interaction (*4*, *28*−*30*). Therefore, a better understanding of the species and levels of aglycons is needed. Species of aglycon are not so diverse, numbering around a few hundred in food polyphenols.

Aglycons of polyphenols can be classified into polycyclic types such as flavonoids, anthraquinones and others, and simple polyphenols. Flavonoids are a large class constructed basically with A and C rings of benzo-1-pyran-4-quinone and a B ring, and further subclassified as flavones (basic structure), flavonols (having a hydroxyl group at the 3-position), isoflavones (B ring binds to the 3-position), flavanones (2−3 bond is saturated), and catechins (C-ring is 1-pyran), chalcones (C-ring is opened), and anthocyanidins (C-ring is 1-pyran, and 1−2 and 3−4 bonds are unsaturated). They generally have a variety of substitutions involving hydroxyl and/or methoxyl groups. Anthraquinones are known to comprise around 20 species, such as alizarin, rhein, and emodin (*31*). The other polycyclic types are caffeine, sesamol, ellagic acid, and so on. Simple polyphenols include two subclasses, cinnamic acids, such as coumaric acid, ferulic acid, and caffeic acid, and benzoic acids, such as protocatechuic acid, gallic acid, and vanillic acid.

Several groups have proposed methods for identifying aglycons by high-performance liquid chromatography (HPLC) with a photodiode array detector (*32*−*35*). These methods had not been able to cover all polyphenols, however, because they targeted only a part of the flavonoids. Further, they required

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Table 1. A Library of the Analytical Characteristics of Polyphenols

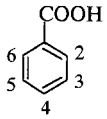
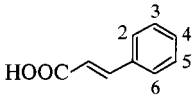
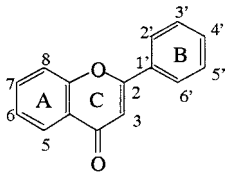
polyphenols	λ_{max} (nm)	t_R[a] (min)	calibration		
			determining[b] (nm)	slope[c] ($\times 10^{-4}$)	limit[d] (pmol)
Simple Polyphenols					
					
benzoic acids					
o-hydroxybenzoic acid (2-OH)	273, 321	34.3	250	59.7	190
m-hydroxybenzoic acid (3-OH)	234, 293	16.5	250	19.7	103
p-hydroxybenzoic acid (4-OH)	211sh, 253	13.8	250	3.87	46
protocatechuic acid (3,4-OH)	257, 291	9.7	250	4.77	56
β-resorcylic acid (2,4-OH)	250, 291	10.9	250	8.48	60
vanillic acid (4-OH, 3-OCH_3)	257, 290	16.3	250	5.74	50
gallic acid (3,4,5-OH)	269	5.8	250	17.0	74
cinnamic acids					
o-coumaric acid (2-OH)	213sh, 232sh, 276	21.9	320	4.36	38
m-coumaric acid (3-OH)	211sh, 231sh, 275, 320sh	24.8	320	9.99	54
p-coumaric acid (4-OH)	207sh, 226sh, 291sh, 307	23.3	320	2.36	22
caffeic acid (3,4-OH)	215, 241sh, 291sh, 319	18.4	320	2.07	38
chlorogenic acid (caffeoylquinic acid)	217, 241sh, 294sh, 321	14.1	320	2.02	36
ferulic acid (4-OH, 3-OCH_3)	214, 239sh, 291sh, 325	25.8	320	2.09	33
isoferulic acid (3-OH, 4-OCH_3)	215sh, 239sh, 291sh, 325	26.3	320	2.69	51
Flavonoids					
					
flavones					
flavone (none)	247, 295, 309sh	88.8	280	2.53	32
7,4′-dihydroxyflavone	231sh, 253sh, 307sh, 329	75.7	320	1.53	23
7,3′,4′-trihydroxyflavone	235sh, 253sh, 307sh, 339	60.5	320	2.09	41
chrysin (5,7-OH)	243sh, 267, 311	88.8	320	2.78	36
genkwanin (5,4′-OH, 7-OCH_3)	265, 334	90.5	320	8.40	20
baicalein (5,6,7-OH)	215sh, 274, 321	84.0	320	2.44	42
baicalein-7-*O*-glucuronide (baicalin)	216sh, 276, 315	51.0	320	1.93	64
apigenin (5,7,4′-OH)	265, 290sh, 336	80.8	320	1.99	15
apigenin-6-*C*-glucoside (isovitexin)	269, 334	38.2	320	1.74	50
apigenin-7-*O*-glucoside (apigetrin)	265, 333	50.7	320	1.89	67
apigenin-8-*C*-glucoside (vitexin)	267, 294sh, 334	31.7	320	2.18	44
vitexin-2′′-*O*-rhamnoside	267, 294sh, 336	35.2	320	2.24	62
luteolin (5,7,3′,4′-OH)	252, 265, 290sh, 347	78.9	320	3.03	26
luteolin-6-*C*-glucoside (homoorientin)	212sh, 254, 267, 346	27.5	320	2.57	35
luteolin-7-*O*-glucoside	253, 265, 346	37.1	320	2.85	48
luteolin-8-*C*-glucoside (orientin)	254, 266, 346	26.2	320	2.76	42
luteolin-3′,7-di-*O*-glucoside	240, 266, 338	31.6	320	2.63	74
luteolin-4′-*O*-glucoside	244sh, 265, 290sh, 336	54.9	320	2.34	45
diosmetin (5,7,3′-OH, 4′-OCH_3)	250, 265, 344	84.0	320	2.84	32
diosmetin-7-*O*-rhamnoside (diosmin)	251, 265, 344	61.4	320	2.40	60
chrysoeriol (5,7,4′-OH, 3′-OCH_3)	249, 266, 287sh, 344	83.8	320	2.51	22
5,7-dihydroxy-3′,4′,5′-trimethoxyflavone	269, 305sh, 329	88.3	320	2.25	33
tangeretin (5,6,7,8,4′-OCH_3)	269, 323	91.6	320	1.43	14
gardenin A (5,6,7,8,3′,4′,5′-OCH_3)	247, 256sh, 295, 310sh	88.6	320	2.49	30
sinensetin (5,6,7,3′,4′-OCH_3)	240sh, 267, 329	86.3	320	1.86	39
flavonols					
flavonol (3-OH)	236, 344sh, 305, 341	91.5	370	8.08	75
galangin (3,5,7-OH)	237sh, 264, 307, 355	89.9	370	3.49	46
datiscetin (3,5,7,2′-OH)	257, 304, 346	83.1	370	6.10	112
kaempferol (3,5,7,4′-OH)	264, 292sh, 318sh, 363	82.3	370	1.90	20
kaempferol-3-*O*-glucoside (astragalin)	264, 291sh, 320sh, 344	55.6	370	4.42	41
kaempferol-3-*O*-rutinoside	263, 292sh, 344	58.0	370	5.30	96
kaempferol-7-*O*-neohesperidoside	246, 263, 318sh, 361	53.2	370	2.18	68
morin (3,5,7,2′,4′-OH)	251, 261sh, 354	58.2	370	3.94	120
quercetin (3,5,7,3′,4′-OH)	253, 268sh, 297sh, 368	75.5	370	1.86	30
quercetin-3-*O*-glucoside (isoquercitrin)	253, 263sh, 294sh, 351	41.7	370	3.00	59
quercetin-3-*O*-rutinoside (rutin)	255, 265sh, 294sh, 352	40.6	370	3.06	55
quercetin-3-*O*-rhamnoside (quercitrin)	253, 263sh, 344	56.9	370	5.82	50
robinetin (3,7,3′,4′,5′-OH)	249, 317, 361	34.0	370	1.84	87
isorhamnetin (3,5,7,4′-OH, 3′-OCH_3)	253, 269sh, 302sh, 367	84.2	370	5.04	16
tamarixetin (3,5,7,3′-OH, 4′-OCH_3)	253, 268sh, 296sh, 364	83.8	370	1.68	15
quercetagetin (3,5,6,7,3′,4′,-OH)	257, 273sh, 358	41.0	370	2.59	87
myricetin (3,5,7,3′,4′,5′-OH)	251, 267sh, 300sh, 370	49.3	370	2.63	78
myricetin-3-*O*-rhamnoside (myricitrin)	250sh, 262, 298sh, 349	36.4	370	3.77	84

Table 1. (Continued)

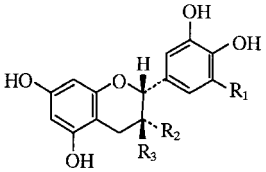
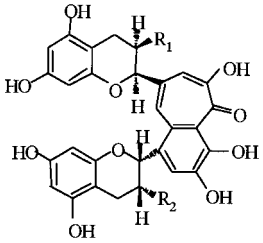
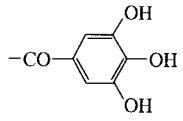
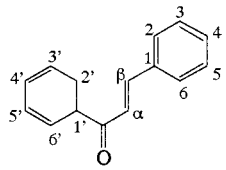
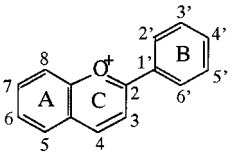
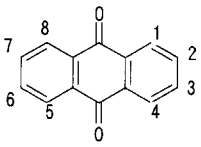
polyphenols	λ_{max} (nm)	t_R[a] (min)	calibration: determining[b] (nm)	calibration: slope[c] ($\times 10^{-4}$)	calibration: limit[d] (pmol)
flavanones (2–3 is saturated)					
naringenin (5,7,4′-OH)	226sh, 288, 331sh	75.2	280	2.63	38
naringenin-7-*O*-rutinoside (naringin)	211sh, 224sh, 281, 326sh	41.3	280	2.20	42
eriodictyol (5,7,3′,4′-OH)	227sh, 286, 332sh	54.4	280	2.10	40
hesperetin (5,7,3′-OH, 4′-OCH_3)	229sh, 286, 333sh	79.4	280	2.28	20
hesperetin-7-*O*-rutinoside (hesperidin)	224sh, 281, 334sh	45.6	280	2.04	50
(+)-taxifolin (3,5,7,3′,4′-OH)	229sh, 287, 333sh	26.7	280	2.33	30
isoflavones (B-ring binds to 3 position)					
daidzein (7,4′-OH)	239sh, 246, 261sh, 300	64.1	250	1.61	52
daidzein-7-*O*-glucoside (daidzin)	248, 258sh, 299	24.9	250	1.70	45
daidzein-8-*C*-glucoside (puerarin)	239sh, 248, 302	20.1	250	1.41	130
genistein (5,7,4′-OH)	259, 329sh	79.8	250	1.34	15
genistein-7-*O*-glucoside (genistin)	259, 325	31.9	250	1.32	34
glycitein (7,4′-OH, 6-OCH_3)	253, 317	73.1	250	1.87	16
glycitein-7-*O*-glucoside (glycitin)	257, 319	25.6	250	1.79	30
biochanin A (5,7-OH, 4′-OCH_3)	259, 329sh	88.8	250	1.34	120
formononetin (7-OH, 4′-OCH_3)	224sh, 246, 261sh, 302	85.1	250	1.63	21
Catechins and Theaflavins					
  G = 					
catechins					
(+)-catechin (R_1 = H, R_2 = H, R_3 = OH)	230sh, 278	13.6	280	11.3	180
(−)-gallocatechin (R_1 = OH, R_2 = H, R_3 = OH)	269	8.1	280	140	290
(−)-catechin gallate (R_1 = H, R_2 = H, R_3 = OG)	276	26.1	280	2.56	42
(−)-gallocatechin gallate (R_1 = OH, R_2 = H, R_3 = OG)	273	19.7	280	3.62	68
(−)-epicatechin (R_1 = H, R_2 = OH, R_3 = H)	229sh, 277	18.4	280	11.5	94
(−)-epigallocatechin (R_1 = OH, R_2 = OH, R_3 = H)	229sh, 269	13.1	280	65.8	360
(−)-epicatechin gallate (R_1 = H, R_2 = OG, R_3 = H)	276	22.9	280	2.12	30
(−)-epigallocatechin gallate (R_1 = OH, R_2 = OG, R_3 = H)	273	17.0	280	3.24	80
theaflavins					
theaflavine (R_1 = OH, R_2 = OH)	229sh, 267, 370, 446	80.8	280	1.92	18
theaflavin-3-gallate (R_1 = OG, R_2 = OH)	225sh, 269, 370, 446	80.4	280	2.26	20
theaflavin-3′-gallate (R_1 = OH, R_2 = OG)	225sh, 273, 370, 446	81.2	280	1.68	14
theaflavin-3,3′-digallate (R_1 = OG, R_2 = OG)	227sh, 273, 370, 446	81.3	280	1.28	10
Chalcones					
					
chalcone (none)	227sh, 309	92.1	320	1.06	30
isoliquiritigenin (4,2′,4′-OH)	255sh, 298sh, 367	84.2	320	2.86	39
butein (3,4,2′,4′-OH)	259, 305sh, 378	79.2	320	4.02	38
phloretin (α–β bond is saturated, 4,2′,4′,6′-OH)	225sh, 285	80.3	320	11.5	81
Anthocyanins					
					
pelargonidin (3,5,7,4′-OH)	267, 325sh, 409, 503	47.7	510	9.03	30
cyanidin (3,5,7,3′,4′-OH)	276, 320sh, 503	36.3	510	13.3	39
cyanidin-3-*O*-rutinoside	229sh, 278, 424sh, 503	17.4	510	8.87	57
delphinidin (3,5,7,3′,4′,5′-OH)	271, 336sh, 429, 521	28.2	510	9.94	27
Anthraquinones					
					
anthraquinone (none)	251, 270sh, 325	88.5	250	0.84	26
alizarin (1,2-OH)	246, 278, 423	84.5	250	1.48	46
purpurin (1,2,4-OH)	253, 288, 472	89.2	250	1.94	60
emodin (1,6,8-OH, 3-CH_3)	221sh, 251, 265, 285, 434	83.4	250	2.93	12
rhein (1,8-OH, 3-COOH)	228, 257, 424	85.3	250	1.14	5.7

Table 1 (Continued)

		Others			
caffeine	231sh, 271	16.7	280	4.17	30
sesamol	230, 293	19.2	280	1.64	24
ellagic acid	251, 300sh, 358	43.1	250	0.84	12

[a] Retention times. [b] Wavelength for the determination. [c] Calibration curves $y = ax$, where a is the slope, x is the peak area, and y is the concentration (μM). [d] Determination limit when 10 μL of sample was analyzed, which was the amount giving a peak 10 times greater than the largest noise peak in the area.

pretreatment by hydrolysis, although this produces a loss of content due to the decomposition and polymerization of polyphenols. For example, under optical conditions, hydrolysis led to an underestimation of up to 50% of the true polyphenol level in food (*32*). Additionally, the results were affected by temperature, time, other components, and so on. Schieber et al. (*36*) improved the HPLC method to detect simultaneously aglycons and their glycosides without hydrolysis. However, the method could detect only one aglycon, quercetin.

Since the identification of aglycon species is essential, the direct determination of forms such as glycosides in foods without hydrolysis is needed. In the present study, we developed a HPLC method to quantify and identify every polyphenol, including glycoside and aglycon forms, in vegetables, fruits, and teas.

MATERIALS AND METHODS

Chemicals. The standard chemicals used to make the library were purchased as follows: most of the flavonoids were from Extrasynthèse (Genay, France), flavone was from Nacalai Tesque (Kyoto, Japan), and flavonol was from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Eight catechins were from Kurita Kogyo (Tokyo, Japan), and theaflavins were kindly provided by Ito-En, Ltd. (Tokyo, Japan). Simple polyphenols were high-grade commercially available products. These chemicals were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM after examination of their purity by nuclear magnetic resonance (NMR) spectral analysis with a Bruker AC-250 (Bruker Analytik GMBH) and stored at −20 °C in the dark for up to 3 months. After dilution of these polyphenol solutions to a range from 1.0 to 1000 μM with DMSO, calibration curves were made by HPLC with a photodiode array detector. Water was distilled twice, and all other reagents were of the highest grade available.

Vegetables, Fruits, and Teas. Fresh vegetables and fruits were obtained from local markets in Kobe City independently several times. The edible portions (100 g) were taken randomly from several individual samples and washed with tap water. After being chopped, they were homogenized in liquid nitrogen with a homogenizer (Nihonseiki Kaisha Co., Ltd., Osaka, Japan). Teas, cacao, and coffee beans from local groceries in Kobe City were powdered with a coffee mill. The homogenate and powder were lyophilized at 0.2 Pa for 48 h and stored at 4 °C in a desiccator before use.

Extraction of Polyphenols. The stored powders (50 mg) were extracted with 2 mL of 90% methanol containing 0.5% acetic acid, after adding 50 nmol of flavone in DMSO. Flavone was used mostly as an internal standard, because vegetables, fruits, and teas have been found to rarely contain flavone. When food samples gave a flavone peak on the HPLC, the internal standard was replaced with another, flavonol or chalcone. The solution was allowed to stand in a sonicater for 1 min, and the supernatant was recovered by centrifugation at 3000 rpm for 10 min. After extraction three times, the extracts were dried with a centrifugal concentrator (VC-96N, Taitec Co., Saitama, Japan). The residues were dissolved in 0.5 mL of DMSO and filtered through a Millex-LG 0.2-μm membrane filter (Millipore Co., Bedford, MA) before the HPLC analysis. The treatment was repeated independently three times or more until the variation in the recoveries calculated with the internal standard was less than 5%.

HPLC. The HPLC system employed was a Hitachi HPLC series D-7000 (Tokyo, Japan) equipped with Hitachi model D-7000 chromatography data station software, autosampler D-7200, column oven D-7300, and diode array detection system D-7450 to monitor at all wavelengths from 200 to 600 nm. For the column, Capcell pak C18 UG120 (250 × 4.6 mm i.d., S-5, 5 μm, Shiseido Co., Ltd., Tokyo, Japan), joined with a guard column (10 × 4.0 mm i.d.), was used at 35 °C. Gradient elution was performed with solution A, composed of 50 mM sodium phosphate (pH 3.3) and 10% methanol, and solution B, comprising 70% methanol, delivered at a flow rate of 1.0 mL/min as follows: initially 100% of solution A; for the next 15 min, 70% A; for another 30 min, 65% A; for another 20 min, 60% A; for another 5 min, 50% A; and finally 0% A for 25 min. The injection volume for the extract was 10 μL.

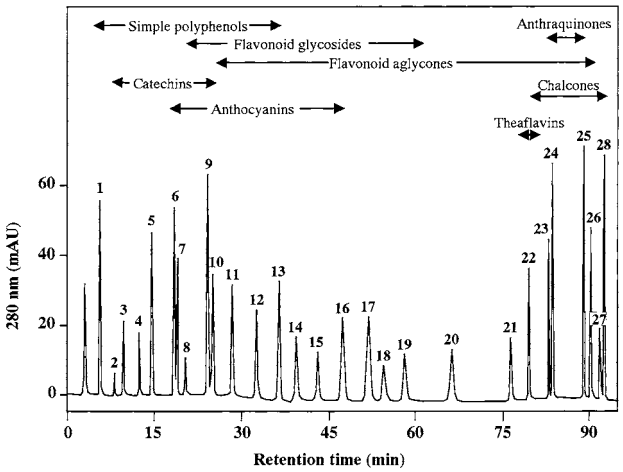


Figure 1. Typical HPLC profile for polyphenols. Numbers show the following standard chemicals: 1, gallic acid; 2, gallocatechin; 3, protocatechuic acid; 4, β-resorcylic acid; 5, chlorogenic acid; 6, caffeic acid; 7, epigallocatechin gallate; 8, sesamol; 9, *p*-coumaric acid; 10, daidzein-7-*O*-glucoside; 11, catechin gallate; 12, luteolin-3′,7-*O*-diglucoside; 13, *o*-coumaric acid; 14, luteolin-7-*O*-glucoside; 15, quercetin-3-*O*-rutinoside; 16, hesperetin-7-*O*-rutinoside; 17, myricetin; 18, apigenin-7-*O*-glucoside; 19, kaempferol-3-*O*-glucoside; 20, daidzein; 21, quercetin; 22, luteolin; 23, kaempferol; 24, apigenin; 25, flavone; 26, galangin; 27, flavonol; 28, chalcone. The other polyphenols were eluted in the positions shown with arrows.

Polyphenol Analyses. First, we made a library, comprising retention times on HPLC and spectra of aglycons, with a diode array detector for 100 standard chemicals and constructed the respective calibration curves, as shown in **Table 1**. The food extract was then analyzed using the same HPLC system. The detected polyphenol peaks were first compared with respect to retention time with those in the library, and next the aglycons were identified by comparison with spectra of standard chemicals. When the detected polyphenol did not coincide in terms of retention time with any of the standards, the food samples were subjected to hydrolysis as described in the next section and analyzed again by HPLC. Referring to the non-hydrolysis chromatographs, sample peaks were identified on the basis of the retention times and spectra for aglycons in the library, because almost all naturally occurring aglycons were covered. In rare cases, more information about the structure was required, and the identification was confirmed with a HPLC−mass spectrometer (LC/MS M-1200H, Hitachi) under atmospheric pressure with chemical ionization and ionizing at +30 eV.

Quantitative analysis was performed by chromatography of the non-hydrolyzed samples. Among polyphenol glycosides, those found in the library as related with aglycons were determined from the absorbance

Table 2. Recovery of Polyphenol Standards Added to Radish Root

polyphenol standard	recovery (%)[a]	polyphenol standard	recovery (%)[a]	polyphenol standard	recovery (%)[a]
		Simple Polyphenols			
protocatechuic acid	75 ± 2	*p*-hydroxycinnamic acid	68 ± 1	chlorogenic acid	70 ± 4
o-hydroxycinnamic acid	78 ± 6	gallic acid	87 ± 3	caffeic acid	77 ± 9
		Flavonoids			
apigenin	78 ± 5	quercetin	83 ± 3	naringenin	79 ± 3
apigenin-7-*O*-glucoside	73 ± 3	quercetin-3-*O*-rutinoside	77 ± 2	naringenin-7-*O*-rutinoside	77 ± 5
luteolin	78 ± 7	myricetin	73 ± 2	flavone	81 ± 5
luteolin-7-*O*-glucoside	80 ± 4	myricetin-3-*O*-rhamnoside	92 ± 8	flavonol	76 ± 3
kaempferol	81 ± 2	daidzein	86 ± 3	chalcone	83 ± 3
kaempfenol-3-*O*-glucoside	81 ± 7	daidzein-7-*O*-glucoside	74 ± 4		

[a] Fifty nanomoles each of the standard polyphenols was added to 50 mg of radish root powder and then extracted as described in the Materials and Methods. Values are the mean ± SE ($n = 6$).

of the glycosides, and the glycosides not in the library were determined from the absorbance of the aglycons. For instance, the absorbance of quercetin-3-*O*-rutinoside (rutin) was used for all quercetin glycosides, and that of caffeic acid was used for cinnamic acid glycosides. The calibration curves were constructed with the specific wavelengths of standard chemicals: 250 nm for benzoic acids and isoflavones; 280 nm for flavanones, catechins, caffeine, phloretin, ellagic acid, and flavone; 320 nm for cinnamic acids, flavones other than flavone, and chalcones; 370 nm for flavonols; and 510 nm for anthocyanins, as shown in **Table 1**.

Hydrolysis. When aglycon profiles were required for the identification of glycosides, the stored food powder was subjected to hydrolysis by a modification of the method of Hertog et al. (*32*). Fifty milligrams of powder was placed in a test tube with a rubber cap and mixed with 4 mL of 62.5% aqueous methanol containing 0.5 mg/mL of *tert*-butylhydroquinone and 1 mL of 2 N HCl. The rubber cap was pinholed, the tube was heated at 90 °C for 2 h, and the sample was then extracted with two volumes of ethyl acetate. The extract was dried under a nitrogen gas stream, dissolved in 0.5 mL of DMSO, filtered through a 0.2-μm filter, and analyzed by HPLC.

RESULTS

HPLC Library. Every standard chemical gave an almost linear calibration curve through the zero point. The slopes and determination limits are listed in **Table 1**. Anthraquinone rhein was the most sensitive in the present system, determined at up to 5.7 pmol. (−)-Epigallocatechin was the most insensitive, with a determination limit of 360 pmol.

The present HPLC system was able to detect all of the chemicals in **Table 1** as the respective single peaks with good resolution. **Figure 1** shows a typical profile, with 28 chemicals, at a concentration of 1 nmol each, which are abundant polyphenols in food (*37*, *38*). Simple polyphenols were eluted with retention times between 5.8 and 34.3 min; catechins between 8.1 and 26.1 min; anthocyanins between 17.4 and 47.7 min; glycoside forms of flavones, flavonols, isoflavones, and flavanones between 20.1 and 61.4 min; their aglycon forms between 26.7 and 91.6 min; anthraquinones between 83.4 and 89.2 min; chalcones between 79.2 and 92.1 min; and theaflavins between 80.4 and 81.3 min. All chemicals in **Table 1** were eluted in 95 min.

Recovery with Extraction. The recovery with the extraction was examined using Japanese radish root, which is known to contain few polyphenols (*37*, *38*). Fifty nanomoles each of 23 standard chemicals was added to the 50 mg powder of radish root and extracted and analyzed by HPLC as described in the Materials and Methods (**Table 2**). The recovery of chemicals when determined independently three times was in the range 68−92%, and the variance was 1−9%. The recovery and the low variance were considered to be sufficient to determine food polyphenols quantitatively.

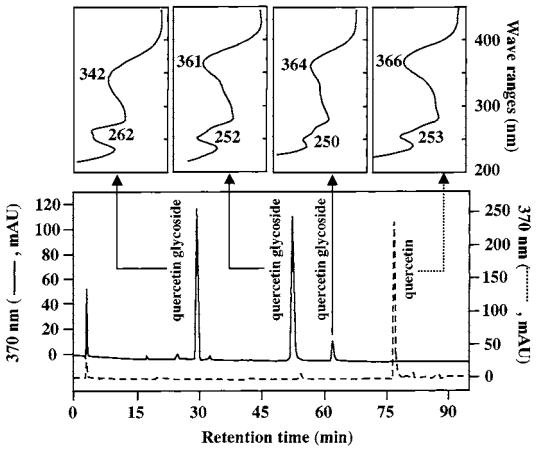


Figure 2. Analyses for onion polyphenols without (solid line) and with (dashed line) hydrolysis.

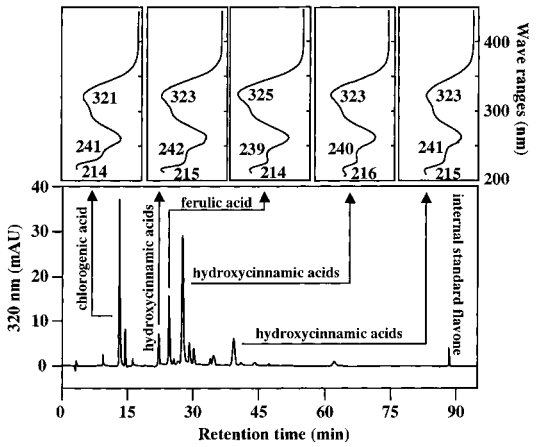


Figure 3. Analysis of burdock polyphenols.

Next, onion was employed, which contains quercetin glycosides at high levels and has been used as a model for many analyses (*39*−*43*). **Figure 2** shows the results of HPLC and spectra of three major peaks. The retention times did not coincide with those for any of the quercetin glycosides in the library (isoquercitrin, rutin, and quercitrin), but every photodiode array spectrum was very similar to that of quercetin aglycon. The onion powder was then hydrolyzed and analyzed again. Hydrolysis gave a single peak at a retention time of 75.5 min, which coincided with standard quercetin in retention time and spectra. This meant that all three glycosides consisted of

Table 3. Polyphenols Contents of Plant Foodstuffs

food (scientific name)	polyphenol content (μmol/100 g fresh edible part)[a]			
	flavonoids		simple polyphenols	
	Vegetables			
root				
carrot (*Daucus carota* L.)		ud	cinnamic acids	0.5–0.6
burdock (*Arctium lappa* L.)		ud	chlorogenic acid	38–178
			ferulic acid	38–100
			cinnamic acids	120–532
radish (*Raphanus sativus* L.)		ud	cinnamic acids	5.8–13
turnip (*Brassica campestris* L. var. *glabra Kitam*.)		ud	cinnamic acids	8
tuber				
potato (*Solanum tuberosum* L.)		ud	chlorogenic acid	1.9
			cinnamic acids	18
sweet potato (*Ipomoea batatas* L.)		ud	cinnamic acids	54
Chinese yam (*Dioscorea opposita* Thunb.)		ud		ud
bulb				
onion (*Allium cepa* L.)	quercetin glycosides	92–178		ud
	quercetin	1.2–1.9		
petiole				
taro (*Colocasia esculenta* L.)	anthocyanins	7.4		ud
	rutin	7.4		
	quercetin glycosides	2.1		
leaf				
cabbage (*Brassica oleracea* var. *capitata* L.)	quercetin	0.4	caffeic acid	7.1
	luteolin glycosides	1.2	chlorogenic acid	11.1
	kaempferol glycosides	1.6		
celery (*Apium graveolens* L.)	apigenin	5.3–16	chlorogenic acid	17–50
	apigenin glycosides	18–51	cinnamic acids	1.6–2.5
	luteolin glycosides	7.1–21		
	chrysoeriol glycosides	13–38		
Chinese cabbage (*Brassica campestris* var. *perviridis* L.)	kaempferol glycosides	54–115	caffeic acid	8.6–22
			chlorogenic acid	13–28
			cinnamic acids	1.9–26
Chinese chive (*Allium tuberosum* Rottle ex Spreng.)	kaempferol glycosides	16–67	ferulic acid	1.5–7.2
			cinnamic acids	13–22
coriander (*Coriandrum sativum* L.)	rutin	180	cinnamic acids	100
	quercetin glycosides	48		
garland chrysanthemum (*Chrysanthemum coronarium* L.)		ud	chlorogenic acid	1.7–3.5
			cinnamic acids	59–217
Indian spinach (*Basella rubra* L.)	apigenin-6-*C*-glucoside	7.8–12	caffeic acid	3.6–5.1
	apigenin-8-*C*-glucoside	216–336		
lettuce (*Lactuca sativa* L.)	quercetin glycosides	1.7–4.8	caffeic acid	16–86
			chlorogenic acid	3.0–82
mizuna (*Elatostema umbellatum* Blume var. *majus*)	kaempferol glycosides	ud–6.66	caffeic acids	15.3–19.8
	quercetin glycosides	ud–13.3	ferulic acid	7.17–9.42
	isorhamnetin	ud–1.53	isoferulic acid	ud–3.39
mugwort (*Artemisia princeps* Pamp.)		ud	protocatechuic	5.2
			acid	383
			2-hydroxybenzoic	6.8
			acid	7.6
			benzoic acids	
			cinnamic acids	
nalta jute (*Corchorus olitorius* L.)	kaempferol glycosides	8.5–26	cinnamic acids	80.5–729
	rutin	12.5–37.3		
	isoquercitrin	18.1–96.9		
pak choi (*Brassica campestris* var. *chinensis* L.)	kaempferol glycosides	53–133	caffeic acid	4.0–13
			chlorogenic acid	8.9–32
			cinnamic acids	11–44
parsley (*Petroselinum crispum* Nym. ex A.W. Hill.)	apigenin	8.6–331		ud
	apigenin glycosides	235–873		
	kaempferol glycosides	7.8–17		
perilla (red) (*Perilla frutescens* Britt. var. *crispa*)		ud	caffeic acids	1410–1550
quig gin cai (*Brassica campestris* var. *chinensis* L.)	kaempferol glycosides	24–101	chlorogenic acid	5.4–19
			cinnamic acids	15–36
radish (*Raphanus sativus* L.)	quercetin glycosides	233		ud
	kaempferol glycosides	27		
radish (maturity) (*Raphanus sativus* L.)	kaempferol glycosides	48–123	cinnamic acids	168–229
spinach (*Spinacia oleracea* L.)	patuletin glycosides	14.3–26.6	cinnamic acids	1.48–2.89
	spinacetin glycosides	22.2–78.0	chlorogenic acid	1.91–2.59
	5,3′-dihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-glucronide methyl ester (TMMG)	59.8–87.7		
	5-hydroxy-3,3′- dimethoxy-6:7-methylenedioxyflavone-4′-glucronide methyl ester (DDMG)	19.8–27.1		
turnip (*Brassica campestris* L. var. glavra.)	kaempferol glycosides	58	cinnamic acids	34
water dropwort (*Oenanthe javanica* DC.)	quercetin glycosides	16–108	caffeic acid	2.2–14
	isorhamnetin glycosides	65–129	chlorogenic acid	31–62
			ferulic acid	38–178
			cinnamic acids	3.7–10
Welsh onion (*Allium fistulosum* L.) curd or sprout	kaempferol glycosides	79.1–95.4	caffeic acids	8.8–10
asparagus (*Asparagus officinalis* L.)	quercetin glycosides	7.7–95	caffeic acid	1.3–5.7
			chlorogenic acid	9.7–24
			cinnamic acids	1.7–16

Table 3. (Continued)

food (scientific name)	polyphenol content (μmol/100 g fresh edible part)[a]			
	flavonoids		simple polyphenols	
broccoli (*Brassica oleracea* var. *botrytis* L.)	luteolin	13.3	caffeic acid	9.4
	luteolin glycosides	0.6	chlorogenic acid	2.8
	kaempferol glycosides	6.3		
cauliflower (*Brassica oleracea* var. *botrytis* L.)		ud	cinnamic acids	8
fruit				
bell pepper (green) (*Capsicum grossum* L.)	quercetin glycosides	17–23	caffeic acids	4.7–17
	luteolin glycosides	13–37		
bell pepper (maturity) (*Capsicum grossum* L.)	luteolin glycosides	6.3–14		ud
bell pepper (pimento) (*Capsicum grossum* L.)	luteolin glycosides	15–42	chlorogenic acid	34–133
			cinnamic acids	93–280
cacao (*Theobroma cacao* L.)	(+)-catechin	305	caffeine	1084
	(–)-gallocatechin	27000		
	(–)-epicatechin	342		
	(–)-epigallocatechin	512		
corn (*Zae mays* L.)		ud	benzoic acid	40–114
			cinnamic acid	2–27
			ferulic acid	3–12
tomato (*Lycopersicon esculentum* Mill.)	quercetin	0.1	caffeic acid	5.4
	myricetin	1.4	chlorogenic acid	17.9
	myricitrin	1.5		
eggplant (*Solanum melongena* L.)	anthocyanins	229–364	caffeic acid	2.2–14
			chlorogenic acid	31–62
			ferulic acid	38–180
			cinnamic acids	3.7–10
okura (*Abelmoschus esculentus* L.)	quercetin glycosides	65–114		ud
bean and pea				
coffee bean *(Coffea* L.*)*		ud	caffeic acid	166
			chlorogenic acid	698
			caffeine	4032
			cinnamic acids	1350
common bean (*Phaseolus vulgaris* L.)	kaempferol glycosides	4.2–20		ud
soybean (*Glicine max* L.)	genistein	50		ud
	daizein glycosides	478		
	genistein glycosides	346		
black soybean (*Glycine max* L.)	genistein	70	protocatechuic	2.0
	daizein glycosides	263	acid	
	genistein glycosides	290		
	(–)-epicatechin	129		
	anthocyanins	18		
carob (dry) (*Ceratonia siliqua* L.)	quercetin glycosides	231	gallic acid	3540
	(+)-catechin	175	ellagic acid	84
	(–)-epicatechin gallate	68		
	(–)-epigallocatechin gallate	239		
garden pea (*Pisum sativum* L.)	quercetin glycosides	63		ud
	Fruits			
citrus				
grapefruit (*Citrus paradisi* Macf.)	naringenin	4.6–27	caffeic acid	2.1–5.8
	naringenin glycosides	152–438	cinnamic acids	16–27
	apigenin glycosides	6.7–18		
large round kumquat (*Fortunella crassifolia* Swingle)	naringenin glycosides	211		ud
	apigenin glycosides	81		
lemon (*Citrus limon* Burm.)	hesperetin glycosides	135–318	caffeic acid	1.2–2.5
	apigenin glycosides	5.0–12		
	quercetin glycosides	2.8–4.8		
	diosmetin glycosides	40–56		
orange (*Citrus unshiu* Mar.)	hesperidin	148		ud
	naringenin glycosides	167		
others				
apple (*Malus pumila* Mill.)	quercetin glycosides	8.0–13	chlorogenic acid	4.8–35
	(+)-catechin	ud–44	cinnamic acids	0.8
	(–)-epicatechin	ud–60		
	(–)-epigallocatechin	ud–223		
highbush blueberry (*Vaccinium australe* Small)	quercetin glycosides	ud–23	chlorogenic acid	273–325
	anthocyanins	168–471	cinnamic acids	21
Japanese pear (*Pyrus pyrifolia* Nakai)		ud	cinnamic acids	6.6
kiwi fruit (*Actinidia chinensis* Planch)		ud	cinnamic acids	4.6–5.3
loquat (*Eriobotrya japonica* Lindl.)		ud	caffeic acid	16
			chlorogenic acid	250
			cinnamic acids	33
peach (*Prunus persica* L.)	quercetin glycosides	2.8–4.3	chlorogenic acid	12–15
	catechins	29–93	cinnamic acids	13–18
pear (*Pyrus communis* L.)		ud	chlorogenic acid	2.6
			cinnamic acids	0.7
sweet cherry (*Prunus avium* Moench)	quercetin glycosides	13	cinnamic acids	317
	anthocyanins	81		

[a] The data are expressed mostly as the range between lower and higher cases, because the foods were obtained in different seasons, May–July, August, September–October, or November–December. Values without a range are for foods cultured in one season. All food was analyzed independently three times with duplicate determinations, and all values are the mean. "ud" means under the detection limit when the extract from up to 5 g of fresh food was analyzed by HPLC. Values are expressed as aglycon amounts. In the polyphenol names, the singular form is found in the library (**Table 1**), and the plural form is one whose aglycon is detected as several forms of glycosides.

Table 4. Polyphenols in Teas (*Camellia sinensis* L.)

	polyphenols content (μmol/100 g leaf)[a]					polyphenols content (μmol/100 g leaf)[a]			
	green tea Shizuoka, Japan		oolong tea	black tea		green tea Shizuoka, Japan		oolong tea	black tea
	gyokuro	sencha	China	Kenya		gyokuro	sencha	China	Kenya
(+)-catechin	872	278	207	158	kaempferol-3-*O*-glucoside	268	182	83	305
(−)-gallocatechin	227	1460	999	ud	kaempferol-3-*O*-rutinoside	12	ud	60	253
(−)-catechin gallate	32	ud	45	115	kaempferol glycosides	250	259	43	115
(−)-gallocatechin gallate	447	375	297	271	quercetin-3-*O*-rhamnoside	30	116	139	633
(−)-epicatechin	2360	5800	665	2010	quercetin glycosides	150	769	198	ud
(−)-epigallocatechin	8060	17900	4910	919	myricetin-3-*O*-rutinoside	98	517	208	208
(−)-epicatechin gallate	1400	2350	894	823	isovitexin	45	88	ud	ud
(−)-epigallocatechin gallate	9170	14900	5380	1020	gallic acid	154	254	1330	1790
theaflavine	ud	ud	27	310	caffeine	14700	13500	11300	13900
theaflavin-3-gallate	ud	ud	26	430					
theaflavin-3′-gallate and theaflavin-3,3′-digallate[b]	ud	ud	70	960					

[a] Analyzed independently three times with duplicate determinations. "ud" means under the detection limit. [b] Theaflavin-3′-gallate and theaflavin-3,3′-digallate were eluted at a similar position and have the same spectrum under the present analytical conditions.

Table 5. Food Sources for Polyphenols

polyphenol classes	examples	food
simple polyphenols	chlorogenic, caffeic, ferulic, and gallic acids	widely distributed, especially in root vegetables
glycosides of flavones and flavonols	apigenin, luteolin, quercetin, kaempferol, and myricetin glycosides	mainly in leaf vegetables
aglycons of flavones and flavonols	apigenin, luteolin, and galangin	parsley, celery, broccoli, and herbs
isoflavones	genistein, daidzein, and its glycosides	soybean
flavanones	naringenin and hesperetin glycosides	citrus fruits
catechins	epigallocatechin, epigallocatechin gallate, and gallocatechin	teas and cacao bean
anthocyanins	anthocyanins	magenta colored foods (eggplant, black soybean, and blueberry)
anthraquinones	emodin, chrysophanol, and rhein	Chinese medicinal plants

quercetin aglycon. We then examined them as quercetin glycosides with the calibration curve of rutin. The sum of the amounts of the three peaks as aglycon quercetin was 117 ± 2.3 μmol/100 g fresh edible part (353 ± 6.9 mg of quercetin/kg). The reproducibility was good, with 2.5% variance when the extraction and analysis were done independently six times. Other groups have reported the quercein content of onion to be 284−486 (*39*, *40*) or 185−634 mg/kg (*41*). The present method was considered suitable for analyzing food polyphenols.

This method was applied to burdock, which is often consumed in Japan, although little is known about its polyphenols content. Two of the detected peaks coincided with standard chlorogenic and ferulic acids in retention times and spectra, and the other major peak showed a spectrum typical for cinnamic acids (**Figure 3**). Thus, burdock contained only simple polyphenols: 178 ± 7.7 μmol of chlorogenic acid, 100 ± 4.3 μmol of ferulic acid, and 532 ± 13.9 μmol/100 g fresh burdock as the sum total of cinnamic acid derivatives. The variance was 4.3% in six determinations.

Determination of Polyphenols in Foods. Using the present method, 59 plant foods (**Table 3**) and three kinds of teas (**Table 4**) were analyzed for polyphenol content and class. The foods were obtained from city markets, and the contents were expressed as a range of the mean when the foods were cultured in various seasons, and as the mean without a range when the foods were cultured in one season. The polyphenol contents were determined independently three times in duplicate.

Most flavonoids occurred as glycoside forms, and the most abundant aglycons were quercetin and kaempferol. Apigenin was found in parsley, celery, Indian spinach, and lemon. Luteolin occurred in celery and bell peppers. The flavanones, naringenin and hesperetin, were detected only in citrus fruits. Anthocyanins were specific to magenta-colored foods such as eggplant, blueberry, and black soybean. Aglycon forms of flavonoids were minor constituents of vegetables and fruits, whereas they have been known to occur abundantly in herbs and herb-like vegetables such as celery, parsley, peppermint, sage, oregano, and thyme (*44*, *45*). Anthraquinones were not detected in any of the vegetables and fruits, but they are known to be contained in medicinal plants (*31*, *46*).

Interestingly, we detected *C*-glycosides of flavonoids in large amounts in Indian spinach. Generally, the flavonoids in edible plants are *O*-glycoside forms (*47*). Indian spinach included 7.8−12 μmol/100 g of apigenin-6-*C*-glucoside (vitexin) and 216−336 μmol of apigenin-8-*C*-glucoside (isovitexin). The *C*-glycosides were found to remain unchanged after the 2 N HCl hydrolysis.

Table 4 shows the polyphenol contents of green, oolong, and black teas. Compared to the results in **Table 3**, tea leaves contained surprisingly large amounts of polyphenols, especially epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCg). For green tea, two kinds were analyzed: gyokuro, which was shielded from strong sunlight, and sencha, which was cultivated under normal conditions. Among teas, sencha is the richest in EC, EGC, and EGCg. The contents in gyokuro were around 2 times lower, in oolong 3.5 times lower, and in black teas 18 times lower. Theaflavins, which are products of the polymerization of catechin and gallocatechin during the fermentation process (*48*), occurred in oolong and

black teas. These tea polyphenols were infused by 58 ± 20% ($n = 6$) when soaked in 15 volumes of 85 °C water (data not shown).

DISCUSSION

In the present study, we developed a method for determining all the polyphenols in vegetables, fruits, and teas at once with a HPLC system and a photodiode array detector. The method detected the natural forms in plants directly without hydrolysis and has three merits. The first is a high level of accuracy because the decomposition of polyphenols during hydrolysis is avoided, especially for unstable anthocyanins (*32*). The second is the ability to determine aglycons and glycosides separately, since the former differ greatly in bioavailability from the latter (*49*, *50*). The third is the ability to obtain information on simple polyphenol contents simultaneously with other polycyclic polyphenols. Thus, the present method can determine quantitatively individual classes of polyphenols, simple polyphenols, flavones, flavonols, flavanones, catechins, isoflavones, anthocyanidins, chalcones, and anthraquinones including their glycosides.

In the pretreatment involving homogenization in liquid nitrogen, lyophilization, and extraction with 90% methanol, the recovery was 68−92%, depending on the chemicals, and the reproducibility of recovery was good, as the variation was in the range 1−9% for simple polyphenols and 2−7% for flavonoids (**Table 2**). The present recovery and reproducibility were similar to or better than those of other methods (*32*, *34*).

We then analyzed polyphenol classes and levels in vegetables and fruits (**Table 3**). The data for the glycosides were shown as micromoles of aglycon, because the molecular weight of glycoside chains was unknown. Other groups expressed the amounts as milligrams of aglycon (*32*−*35*). The results can be compared after multiplying by the molecular weight of the respective aglycon. Each datum in the present analysis was in the range of those reported by other groups. Additionally, the present analysis provided information on glycoside forms and the amounts of simple polyphenols.

In **Table 5**, we summarize polyphenol characteristics for foods commonly consumed on a daily basis. Root vegetables such as carrot, radish, burdock, and potato contained only simple polyphenols. Leaf vegetables such as cabbage, chive, lettuce, and spinach contained flavones and flavonols mainly in the glycoside form. Among leaf vegetables, celery and parsley are classified as herb vegetables along with peppermint, sage, oregano, and thyme (*44*, *45*). They contain aglycon forms of flavones and flavonols at relatively high levels. These characteristics of polyphenol classes seem to depend on UV exposure, as Li et al. (*51*) and Lois (*52*) reported that plants produce flavonoids with simple polyphenols to protect against UV-B irradiation and accumulate them as glycosides. On the other hand, isoflavones occurred specifically in soybeans, flavanones in citrus, and catechins in teas and cacao. Anthocyanidins were present in magenta-colored foods such as eggplant and blueberry, and anthraquinones were probably one of the bioactive components in Chinese medicinal plants (*31*, *46*).

The present study further demonstrates that simple polyphenols such as cinnamic and benzoic acid derivatives at relatively high levels are more widespread in plant foodstuffs than flavonoids. Hollman and Katan (*53*) reported that flavonoid intakes from plant-derived foods were inversely correlated with the incidence of coronary heart disease but not with cancer risk in epidemiological studies. The analysis did not include the simple polyphenol contents of plant foods. Simple polyphenols have an antioxidative potency to prevent oxidative damage to DNA bases similar to that of flavonoids (*27*). When the data on simple polyphenols are added to the epidemiological analysis, the results may change the correlation between intake and cancer risk.

LITERATURE CITED

(1) Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B. S.; Toshima, H.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.* **1995**, *155*, 381−386.

(2) Meyer, A. S.; Heinonen, M.; Frankel, E. N. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food Chem.* **1998**, *61*, 71−75.

(3) Agullo, G.; Gamet-Payrastre, L.; Manenti, S.; Viala, C.; Remesy, C.; Chap, H.; Payrastre, B. Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. *Biochem. Pharmacol.* **1997**, *53*, 1649−1657.

(4) Ashida, H.; Fukuda, I.; Yamashita, T.; Kanazawa, K. Flavones and flavonols at dietary levels inhibit a transformation of aryl hydrocarbon receptor induced by dioxin. *FEBS Lett.* **2000**, *476*, 213−217.

(5) An, J.; Tzagarakis-Foster, C.; Scharschmidt, T. C.; Lomri, N.; Leitman, D. C. Estrogen receptor beta-selective transcriptional activity and recruitment of coregulators by phytoestrogens. *J. Biol. Chem.* **2001**, *276*, 17808−17814.

(6) Lepley, D. M.; Pelling, J. C. Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin. *Mol. Carcinog.* **1997**, *19*, 74−82.

(7) Xagorari, A.; Papapetropoulos, A.; Mauromatis, A.; Economou, M.; Fotsis, T.; Roussos, C. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 181−187.

(8) Bhatia, N.; Agarwal, R. Detrimental effect of cancer preventive phytochemicals silymarin, genistein and epigallocatechin 3-gallate on epigenetic events in human prostate carcinoma DU145 cells. *Prostate* **2001**, *46*, 98−107.

(9) Frey, R. S.; Li, J.; Singletary, K. W. Effects of genistein on cell proliferation and cell cycle arrest in nonneoplastic human mammary epithelial cells: involvement of Cdc2, p21(waf/cip1), p27(kip1), and Cdc2C expression. *Biochem. Pharmacol.* **2001**, *61*, 979−989.

(10) Nagasaka, Y.; Nakamura, K. Modulation of the heat-induced activation of mitogen-activated protein (MAP) kinase by quercetin. *Biochem. Pharamcol.* **1998**, *56*, 1151−1155.

(11) Kobuchi, H.; Roy, S.; Sen, C. K.; Nguyen, H. G.; Packer, L. Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. *Am. J. Physiol.* **1999**, *277*, C403−C411.

(12) Williamson, G.; Plumb, G. W.; Uda, Y.; Price, K. R.; Rhodes, M. J. C. Dietary quercetin glycosides: antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinone reductase in Hepalclc7 cells. *Carcinogenesis* **1996**, *17*, 2385−2387.

(13) Soriani, M.; Rice-Evans, C.; Tyrrell, R. M. Modulation of the UV-A activation of haem oxygenase, collagenase and cyclooxygenase gene expression by epigallocatechin in human skin cells. *FEBS Lett.* **1998**, *439*, 253−257.

(14) Wadsworth, T. L.; Koop, D. R. Effects of the wine popyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages. *Biochem. Pharmacol.* **1999**, *57*, 941−949.

(15) Liang, Y.-C.; Tsai, S.-H.; Tsai, D.-C.; Lin-Shiau, S.-Y.; Lin, J.-K. Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferator-activated receptor-gamma by flavonoids in mouse macrophages. *FEBS Lett.* **2001**, *496*, 12−18.

(16) Verma, A. K.; Johnson, J. A.; Gould, M. N.; Tanner, M. A. Inhibition of 7,12-dimethylbenz(a)anthracene- and N-nitroso-methylurea-induced rat mammary cancer by dietary flavonol quercetin. *Cancer Res.* **1988**, *48*, 5754−5758.

(17) Wei, H.; Tye, L.; Bresnick, E.; Birt, D. F. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. *Cancer Res.* **1990**, *50*, 0, 499−502.

(18) Tanaka, T.; Kojima, T.; Kawamori, T.; Wang, A.; Suzui, M.; Okamoto, K.; Mori, H. Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeine, ellagic, chlorogenic and ferulic acids. *Carcinogenesis* **1993**, *14*, 1321−1325.

(19) Han, B. S.; Park, C. B.; Takasuka, N.; Naito, A.; Sekine, K.; Nomura, E.; Taniguchi, H.; Tsuno, T.; Tsuda, H. A ferulic acid derivative, ethyl 3-(4′-geranyloxy-3-methoxyphenyl)-2-propenoate, as a new candidate chemopreventive agent for colon carcinogenesis in the rat. *Jpn. J. Cancer Res.* **2001**, *92*, 404−409.

(20) Yang, C. S.; Landau, J. M.; Huang, M. T.; Newmark, H. L. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu. Rev. Nutr.* **2001**, *21*, 381−406.

(21) Ricupero, D. A.; Poliks, C. F.; Rishikof, D. C.; Kuang, P.-P.; Goldstein, R. H. Apigenin decreases expression of the myofibroblast phenotype. *FEBS Lett.* **2001**, *506*, 15−21.

(22) Duffy, S. J.; Keaney, J. F.; Holbrook, M.; Gokce, N.; Swerdloff, P. L.; Frei, B.; Vita, J. A. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation* **2001**, *104*, 151−156.

(23) Yamada, K.; Shoji, K.; Mori, M.; Ueyama, T.; Matsuo, N.; Oka, S.; Nishiyama, K.; Sugano, M. Structure−activity relationship of polyphenols on inhibition of chemical mediator release from rat peritoneal exudates cells. *In Vitro Cell. Dev. Biol.* **1999**, *35*, 169−174.

(24) Herrmann, K. Flavonols and flavones in food plants: a review. *J. Food Technol.* **1976**, *11*, 433−448.

(25) Wollenweber, E.; Dietz, V. H. Occurrence and distribution of free flavonoid aglycones in plants. *Phytochemistry* **1981**, *20*, 869−932.

(26) Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343−355.

(27) Sakakibara, H.; Ashida, H.; Kanazawa, K. A novel method using 8-hydroperoxy-2′-deoxyguanosine formation for evaluating antioxidative potency. *Free Radical Res.* **2002**, *36*, 307−316.

(28) Kanazawa, K.; Yamashita, T.; Ashida, H.; Danno, G. Antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of the cytochrome P450 1A family. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 970−977.

(29) Ferte, J.; Kuhnel, J. M.; Chapuis, G.; Rolland, Y.; Lewin, G.; Schwaller, M. A. Flavonoid-related modulators of multidrug resistance: synthesis, pharmacological activity, and structure−activity relationships. *J. Med. Chem.* **1999**, *42*, 478−489.

(30) Casagrande, F.; Darbon, J. M. Effects of structurally related flavonoids on cell cycle progression of human melanoma cells: regulation of cyclin-dependent kinases CDK2 and CDK1. *Biochem. Pharmacol.* **2001**, *61*, 1205−1215.

(31) Sun, M.; Sakakibara, H.; Ashida, H.; Danno, G.; Kanazawa, K. Cytochrome P4501A1-inhibitory action of antimutagenic anthraquinones in medicinal plants and the structure−activity relationships. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1373−1378.

(32) Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* **1992**, *40* 1591−1598.

(33) Crozier, A.; Jensen, E.; Lean, M. E. J.; McDonald, M. S. Quantitative analysis of flavonoids by reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **1997**, *761*, 315−321.

(34) Häkkinen, S. H.; Kärenlampi, S. O.; Heinonen, I. M.; Mykkänen, H. M.; Törrönen, A. R. HPLC method for screening of flavonoids and phenolic acid in berries. *J. Sci. Food Agric.* **1998**, *77*, 543−551.

(35) Merken, H. M.; Beecher, G. R. Liquid chromatographic method for the separation and quantification of prominent flavonoid aglycones. *J. Chromatogr. A* **2000**, *897*, 177−184.

(36) Schieber, A.; Keller, P.; Carle, R. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *J. Chromatogr. A* **2001**, *910*, 265−273.

(37) Robards, K.; Antolovich, M. Analytical chemistry of fruit bioflavonoids. *Analyst* **1997**, *122*, 11R−34R.

(38) Herrmann, K. On the occurrence of flavonol and flavone glycosides in vegetables. *Z. Lebensm. Unters Forsch.* **1988**, *186*, 1−5.

(39) Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Content of potentially anticarcinogenic flavonoid of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agric. Food Chem.* **1992**, *40*, 2379−2383.

(40) Tsushida, T.; Suzuki, M. Content of flavonol glucosides and some properties of enzymes metabolizing the glucosides in onion. *Jpn. Food Sci. Technol.* **1996**, *43*, 642−649.

(41) Crozier, A.; Lean, M. E.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−595.

(42) Justesen, U.; Knuthsen, P.; Leth, T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *J. Chromatogr. A* **1998**, *799*, 101−110.

(43) Teyssier, C.; Amiot, M. J.; Mondy, N.; Auger, J.; Kahane, R.; Siess, M. H. Effect of onion consumption by rats on hepatic drug-metabolizing enzymes. *Food Chem. Toxicol.* **2001**, *39*, 981−987.

(44) Kanazawa, K.; Kawasaki, H.; Samejima, K.; Ashida, H.; Danno, G. Specific desmutagens (antimutagens) in oregano against a dietary carcinogen, Trp-P-2, are galangin and quercetin. *J. Agric. Food Chem.* **1995**, *43*, 404−409.

(45) Samejima, K.; Kanazawa, K.; Ashida, H.; Danno, G. Luteolin: a strong antimutagens against dietary carcinogen, Trp-P-2, in peppermint, sage, and thyme. *J. Agric. Food Chem.* **1995**, *43*, 410−414.

(46) Lee, H.; Tsai, S.-J. Effect of emodin on cooked-food mutagen activation. *Food Chem. Toxicol.* **1991**, *29*, 765−770.

(47) Markham, K. R. Hydrolysis and the analysis of glycosides. In *Techniques of Flavonoid Identification*; Markham, K. R., Ed.; Academic Press: New York, 1982; pp 52−61.

(48) Owuor, P. O.; Obanda, M. The changes in black tea quality due to variations of plucking standard and fermentation time. *Food Chem.* **1998**, *61*, 435−441.

(49) Hollman, P. C. H.; Katan, M. B. Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed. Pharmacother.* **1997**, *51*, 305−310.

(50) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073S−2085S.

(51) Li, J.; Ou-Lee, T.-M.; Raba, R. R.; Amundson, R. G.; Last, R. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell* **1993**, *5*, 171−179.

(52) Lois, R. Accumulation of UV-absorbing flavonoids induced by UV-B radiation in *Arabidopsis thanliana* L. *Planta* **1994**, *194*, 498−503.
(53) Hollman, P. C. H.; Katan, M. B. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem. Toxicol*. **1999**, *37*, 937−942.

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