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Note

Comprehensive Analysis of Polyphenols in Fruits Consumed in Japan

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Polyphenols, a large group of natural antioxidants, are a versatile group of phytochemicals beneficial for disease prevention. In this study, we comprehensively analyzed polyphenols, catechins, procyanidins, simple polyphenols, anthocyanins and flavonoids, in fruits consumed in Japan by high performance liquid chromatography with photo-diode array and mass spectrometric detection to complete the database of food components.

Keywords: polyphenol, fruits, HPLC, PDA

Introduction

Polyphenols form a large versatile group of phytochemicals that are natural antioxidants found to be potentially beneficial for disease prevention. Epidemiologic studies have recently revealed the association of higher polyphenol intake from fruits and vegetables with decreased risk of cardiovascular diseases (Stoclet *et al.,* 2004; Manach *et al.,* 2005). Furthermore, the regular consumption of certain foods and beverages such as apples, berries, wine, coffee and tea may significantly influence the quantity of antioxidants in a diet (Scalbert and Williamson, 2000). Thus, it is important to determine the amounts and species of polyphenols in foods.

The most recent edition (fifth) of the "Standard Tables of Food Composition in Japan" edited by the Resources Council of the Science and Technology Agency of Japan published in 2000 serves as the database of food components in Japan. It lists the contents, such as proteins, lipids, carbohydrates, minerals and vitamins, of various foods consumed in Japan, but lacks data on polyphenols. For example, fruits are rich in polyphenols, but their polyphenol content is not given. Since the role of polyphenols in foods is gaining attention, we comprehensively analyzed the contents of polyphenols in fruits consumed in Japan to complete the database of food components.

There are over one million natural polyphenols, which generally occur as glycosides, and contain various sugar species with various binding forms (Wollenweber and Dietz, 1981). Aglycons of polyphenols can be generally classified into flavonoids and simple polyphenols. Flavonoids are a family of compounds with a C6-C3-C6 skeleton structure. Flavanols, flavonols and anthocyanins are included in this group. Most of them have been shown to possess antioxidant activity, which depends mainly on the number and position of hydroxyl groups in their structure (Rice-Evans *et al.,* 1996). There are two subgroups of simple polyphenols: benzoic acids (protocatechuic acid, gallic acid, etc.) and cinnamic acids (coumaric acid, caffeic acid, etc.) (Sakakibara *et al.,* 2003).

A high-performance liquid chromatography (HPLC) separation method with photo-diode array (PDA) detection has been proposed to determine and quantify polyphenols in fruits by several groups (Crozier *et al.,* 1997; Merken and Beecher, 2000). Although PDA is a useful technique for characterizing the aglycons of flavonoids, information on the molecular weight of compounds is not obtained from this method. Therefore, in addition to PDA, mass spectrometric (MS) detection technique in HPLC analysis has been applied for the identification of polyphenol compounds (Stobiecki, 2000). In the present study, we also used HPLC-PDA and HPLC-MS analysis to identify and quantify the polyphenols.

Materials and Methods

Materials Catechins were purchased from Sigma (St. Louis, MO, USA), anthocyanins and flavonoids were from Funakoshi (Tokyo, Japan), and simple polyphenols were from Wako Pure Chemicals Industries (Osaka, Japan). These chemicals were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and stored at −20℃ in the dark for up to 3 months. Calibration curves of these polyphenols in solutions ranging from 1.0 to 1000 μM were made by HPLC with PDA.

Extraction of polyphenols from fruits Fresh fruit was obtained from local markets in Shizuoka City or directly

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from the producers. The edible portions were taken randomly from several individual samples and washed with tap water. After being chopped, they were homogenized in liquid nitrogen with a Nissei AM-8 homogenizer (Nihonseiki, Osaka, Japan). The homogenized powders were lyophilized for 48 h and stored at −20℃ until use. Each sample was weighed after lyophilization and the water content was also obtained. The stored powders (50 mg) were extracted with 2 mL of 80% methanol containing 0.5% acetic acid, after adding 50 nmol of flavone in DMSO as an internal standard. The solution was allowed to stand in a sonicator for 1 min, and the supernatant was recovered by centrifugation at 1610 *g* for 10 min. After extraction three times, the extracts were concentrated with evaporator and dried with nitrogen gas. The residues were dissolved in 0.5 mL of 80% methanol or DMSO and filtered with a PTFE 0.45 μm membrane filter (Pall, East Hills, NY, USA) before HPLC analysis.

Total polyphenol contents Total polyphenol contents in the extracted powder from fruits were determined by the Folin-Ciocalteu colorimetric method (Kumazawa *et al.*, 2002). Methanol extracts of the powders (1 mg/mL) were mixed with 1 mL of the Folin-Ciocalteu reagent (Kanto Chemicals, Tokyo) and 1 mL of 10% Na_2CO_3, and the absorbance was measured at 760 nm after 1 h incubation at room temperature. Total polyphenol contents were expressed as μmol/100 g gallic acid equivalents.

HPLC-PDA analysis The HPLC analysis of polyphenols was performed using a Jasco HPLC system (Tokyo) equipped with a PDA detector and a reversed phase column Capcell Pak C18 UG120 (250×4.6 mm i.d., 5 μm; Shiseido, Tokyo, Japan). The flow rate was 1.0 mL/min, the injection volume was 10 μL, and the oven temperature was 30℃. The following solvents were used for analysis of catechins, procyanidins, simple polyphenols and anthocyanins: A, 0.1% trifluoroacetic acid (TFA) in water, and B, methanol. The gradient condition 1: 18% B (0–20 min), 18–22% B (20–25 min), 22% B (25–50 min), 22–100% B (50–80 min), was applied for analysis of catechins, procyanidins and simple polyphenols. The gradient condition 2: 22–50% B (0–50 min), 50–70% B (50–80 min), was used for analysis of anthocyanins. For analysis of flavonoids, solvent A, 1% acetic acid in 10% methanol, and solvent B, 1% acetic acid in 70% methanol were used and the gradient condition 3: 0–30% B (0–15 min), 30–35% B (15–45 min), 35–40% B (45–65 min), 40–50% B (65–70 min), 50–100% B (70–85 min), 100% B (85–95 min), was applied.

Quantification of polyphenols Polyphenols were quantified under the analytical conditions described in HPLC-PDA. Each sample was injected in triplicate, and the standard calibration curves were constructed with the specific wavelengths of standard chemicals: 280 nm for catechins, procyanidins and simple polyphenols; 520 nm for anthocyanins and anthocyanidins; 350 nm for flavones and flavonols; and 290 nm for flavanones.

Results and Discussion

Sakakibara *et al.* (2003) reported a method for simultaneously determining the polyphenols in foodstuffs. However, in the present study, we analyzed the polyphenols in 59 kinds (varieties) of fruits by different three HPLC conditions. Fruits used for the present experiment were purchased from a local supermarket or directly from the producers.

The detected polyphenol peaks from extracts of fruits were compared with respect to retention time with those of standard chemicals, and next the aglycons were identified by comparison with those spectra. When the detected polyphenol did not coincide with any of the standards, the food samples were subjected to hydrolysis or LC/MS analysis. Hydrolysis was performed by the method reported by Sakakibara *et al.* (2003).

Table 1 shows the water content (%), total polyphenol content (μmol/100 g) and polyphenol content (μmol/100 g fresh edible part) of each fruit. Sakakibara *et al.* (2003) examined using Japanese radish root to determine the recovery with extraction, and they obtained recoveries 68–92% for added flavonoids, and the analytical precisions ranged from 1 to 9%. We also performed the recovery with extraction experiment, and obtained the similar results to those of Sakakibara *et al.* (data not shown). Sakakibara *et al.* (2003) used flavone as an internal standard, and they corrected the recovery rate with it. Thus, we also used flavone as an internal standard, and the same data analysis were carried out. The water content of each fruit was similar to that described in the Standard Table of Food Composition in Japan. The Folin-Ciocalteu method we used for determination of total polyphenol contents is interfered by reducing substances (Prior *et al.*, 2005). For example, total polyphenol contents of acerolas show high values caused by the high ascorbic acid content (Hwang *et al.*, 2001).

Catechins, procyanins and simple polyphenols were analyzed using HPLC condition 1. We detected (+)-catechin in atemoya, peach and plum, and (−)-epicatechin in apple, apricot and cherimoya. Although (+)-catechin and (−)-epicatechin have been reported to be in various fruits (Luo *et al.*, 2002; Yilmaz and Toledo, 2004), these compounds were not detected in the present study. This is assumed to be because we analyzed only the edible parts of the fruit not the skin or seed, where these compounds are mostly located.

Large amounts of procyanidins B1, B2 and C1, oligomers of catechins, were detected in atemoyas. Cherimoyas also contained considerable amounts of procyanidins. White sapotes also had high contents of procyanidin B1 and B2, especially the latter. Procyanidins are reported to be detected in various fruits such as apples and grapes (Peng *et al.*, 2001). Although procyanidins as well as catechins exist in the skins and seeds of fruits, we did not analyze polyphenols in the skins or seeds of these fruits. Thus, the contents of procyanidins we obtained were lower than the reported values. Most fruits containing large amounts of catechins and procyanidins belong to the rose family, such as example, apples, peaches and plums.

Of the simple polyphenols, chlorogenic acids were detected in many fruits especially marmelo. Other pears

Table 1-1. Polyphenol contents of fruits.

Food (scientific name)	Water content (%)	Total polyphenol content [a] (μmol/100 g)	Polyphenol content (μmol/100 g fresh edible part): flavonoids		simple polyphenols	
Acerola (*Malpighia glabra*)	90.0	19069	cyanidins [b]	43.1	—	
			total anthocyanins [c]	24.1		
			total anthocyanidins [d]	56.7		
			quercetin glycosides [e]	4.5		
Akebia (*Akebia trifoliata*)						
flesh	77.9	1008	—		—	
peel	86.5	5128	quercetin glycosides [e]	238.1	chlorogenic acid	339.6
					neochlorogenic acid	227.1
Apple (*Malus domestica*)	85.2−87.3	1043	(-)-epicatechin	6.5−9.8	chlorogenic acid	26.6−52.8
			procyanidin B1	4.6−6.9		
			procyanidin B2	12.7−18.2		
			procyanidin C1	2.8−5.4		
Apricot (*Prunus armeniaca*)	92.5	119	rutin	0.2	chlorogenic acid	29.5
					neochlorogenic acid	4.4
Japanese apricot (*Prunus mume*)	91.2	502	(+)-catechin	12.2	caffeic acid	5.3
			(-)-epicatechin	13.4	chlorogenic acid	22.5
			procyanidin B2	6.1	neochlorogenic acid	81.0
			procyanidin C1	2.7		
			rutin	0.3		
Atemoya (*Annona atemoya*)	78.1	4410	(+)-catechin	107.5	—	
			(-)-epicatechin	75.9		
			procyanidin B1	35.0		
			procyanidin B2	58.3		
			procyanidin C1	66.2		
Avocado (*Persea americana*)	66.9	344	—		*p*-coumaric acid	6.5
Banana (*Musa sapientum*)	70.3−79.4	1136	myricetin glycosides [e]	1.0−7.3	—	
Blue-berried honeysuckle (*Lonicera caerulea* subsp. *edulis*)	84.9	2211	(+)-catechin	4.8	chlorogenic acid	86.6
			procyanidin B1	24.3		
			procyanidin B2	5.3		
			cyanidin-3-glucoside	26.0		
			pelargonidin-3-glucoside	2.0		
			cyanidin-3,5-diglucoside	1.8		
			cyanidins [b]	406.0		
			total anthocyanins [c]	65.6		
			total anthocyanidins [d]	423.0		
			rutin	23.4		
			quercetin glycosides [e]	16.2		

Table 1–2. (Continued).

Food (*scientific name*)	Water content (%)	Total polyphenol content [a] (μmol/100 g)	Polyphenol content (μmol/100 g fresh edible part): flavonoids		simple polyphenols	
Blueberry (*Vaccinium* spp.)	84.9−85.9	1104	malvidin-3-galactoside	8.7−18.2	cinnamic acid	1.7
			malvidin-3-glucoside	0.2−8.8	caffeic acid	88.8
			cyanidins [b]	9.1−9.2	chlorogenic acid	57.4−270.6
			delphinidins [b]	27.2−52.1		
			malvidins [b]	79.0−134.0		
			peonidins [b]	1.5−2.7		
			total anthocyanins [c]	75.1−80.1		
			total anthocysnidins [d]	138.0−226.0		
Cherimoya (*Annona cherimola*)	72.5	2612	(-)-epicatechin	172.5	—	
			procyanidin B1	34.2		
			procyanidin B2	130.4		
			procyanidin C1	37.7		
Cherry (*Prunus avium*)						
Japan, sweet type	85.6	481	(+)-catechin	11.6	chlorogenic acid	6.7
			(-)-epicatechin	11.5	neochlorogenic acid	57.8
			procyanidin B1	3.5		
			cyanidin-3-rutinoside	0.5		
			cyanidins [b]	8.1		
			rutin	1.9		
U.S.A., sweet type	81.0−82.8	611	(+)-catechin	13.4−14.6	chlorogenic acid	10.0−16.1
			(-)-epicatechin	6.9−37.0	neochlorogenic acid	51.9−266.2
			procyanidin B1	0.0−12.0		
			cyanidin-3-rutinoside	2.7		
			cyanidins [b]	25.7		
			rutin	1.2−1.9		
Chinese bayberry (*Myrica rubra*)	90.2	772	cyanidin-3-glucoside	0.7−1.1	—	
			myricetin glycosides [e]	16.7		
			quercetin glycosides [e]	1.4		
			rutin	0.3		
Coconut (*Cocos nucifera*) coconut milk	—	9245	—		—	
Date (*Phoenix dactylifera*) dried	15.2	1806	—		—	
Durian (*Durio zibethinus*)	73.3	505	—		—	
Fig (*Ficus carica*) raw	86.8	145	—		chlorogenic acid	1.6
					neochrologenic acid	3.0
dried	27.5	1343	rutin	2.1	neochrologenic acid	22.7
			quercetin glycosides [e]	1.6		
			kaempferol glycosides [e]	0.5		

Table 1–3. (Continued).

Food (*scientific name*)	Water content (%)	Total polyphenol content [a] (µmol/100 g)	Polyphenol content (µmol/100 g fresh edible part) flavonoids		simple polyphenols	
Garambola (*Averrhoa carambola*)	91.2	632	(-)-epicatechin	15.0	—	
			procyanidin B2	18.9		
			procyanidin C1	5.7		
Grape (*Vitis* spp.)						
Delaware unpeeled	78.3	2163	cyanidin-3-glucoside	3.5	—	
			total anthocyanins [c]	5.9		
			quercetin glycosides [e]	1.7		
peeled	78.9	657	—		—	
(Rasins) dried	10.7	3545	quercetin glycosides [e]	2.9	—	
Kyoho unpeeled	78.5	2455	cyanidin-3-rutinoside	2.7	—	
			cyanidin-3-glucoside	1.0		
			malvidin-3,5-diglucoside	24.7		
			malvidin-3-glucoside	3.9		
			total anthocyanins [c]	18.4		
			mirycetin glycosides [e]	9.4		
			quercetin glycosides [e]	4.6		
peeled	80.5	425	—		—	
Guava (*Psidium guajava*)	83.9	2807	—		—	
Japanese persimmon (*Diospyros kaki*)						
nonastringent	83.1	912	—		gallic acid	5.9
astringency removed	82.9	451	—		gallic acid	15.0
dried	19.0	1887	—		gallic acid	239.0
Jujube (*Zizyphus jujuba*) dried	13.2	4160	rutin	3.5	—	
Kiwano (*Cucumis metuliferus*)	90.3	85	—		—	
Kiwifruit (*Actinidia deliciosa*)	83.9	1007	—		—	
Kiwifruit gold	83.3	1743	—		chlorogenic acid	4.5
Lemon (*Citrus limon*)						
whole	83.2	2712	diosmin	134.4	—	
			hesperidin	917.4		
			eriocitrin	367.4		

Table 1-4. (Continued).

Food (*scientific name*)	Water content (%)	Total polyphenol content [a] (μmol/100 g)	Polyphenol content (μmol/100 g fresh edible part) flavonoids		simple polyphenols	
Lemon (*Citrus limon*) fruit juice	—	148	diosmin	12.0	—	
			hesperidin	26.4		
			eriocitrin	17.0		
Lime (*Citrus aurantifolia*) fruit juice	—	310	diosmin	10.2	—	
			hesperidin	44.9		
			eriocitrin	13.4		
Longan (*Euphoria longana*) canned in light syrup	77.1	526	—		—	
Loquot (*Eriobotrya japonica*)	90.5	1064	(-)-epicatechin	15.2	chlorognic acid	95.2
			procyanidin B2	6.6	neochlorogenic acid	89.7
Lychee (*Litchi chinensis*)	80.4	1894	rutin	0.7	—	
			quercetin glycosides [e]	4.1		
Mango (*Mangifera indica*)	85.2	706	—		gallic acid	0.8
Apple mangoe	82.9	547	—		gallic acid	7.5
Mangosteen (*Garcinia mangostana*)	82.9	603	—		—	
Melon (*Cucumis melo*)						
green house culture	90.7	137	—		—	
open culture	85.8	327	—		—	
Oriental melon (*C. melo* var. *makuwa*)	93.1	304	—		—	
Oleaster (*Elaeagnus* spp.)	86.6	4999	—		—	
Oroblanco (*Citrus paradisi*) juice sacs	88.7	442	naringin	51.9	cryptochlorogenic acid	4.1
			narirutin	107.0		
			hesperidin	0.8		
Papaya (*Carica papaya*)						
early ripening type	88.9	1175	—		—	
normal ripening type	90.7	902	—		—	
Passion fruit (*Passiflora edulis*)	85.0	285	—		—	
Peache (*Purunus persica*)	89.3−89.6	431	(+)-catechin	10.1−11.9	chlorogenic acid	6.3−7.0
			procyanidin B1	4.1−5.0	neochlorogenic acid	10.2−12.0

Table 1-5. (Continued).

Food (*scientific name*)	Water content (%)	Total polyphenol content [a] (μmol/100 g)	Polyphenol content (μmol/100 g fresh edible part): flavonoids		simple polyphenols	
Nectarine (*Purunus persica* var. *nucipersica*)	90.4	563	(+)-catechin	12.6	chlorogenic acid	31.3
					neochlorogenic acid	14.1
Pear						
Chinese pear (*Pyrus ussuriensis*)	87.4	547	—		chlorogenic acid	21.0
European pear (*Pyrus communis*)	85.9−86.2	580	(-)-epicatechin	0.0−4.4	chlorogenic acid	6.6−18.1
			procyanidin B2	0.0−6.2		
Japanese pear (*Pyrus pyrifolia* var. *culta*)	85.8−88.2	197	—		chlorogenic acid	3.1−7.2
Marmelo (*Pyrus cydonia*)	79.1	3087	—		chlorogenic acid	91.1
					neochlorogenic acid	76.7
Pineapple (*Ananas comos*	85.4	532	—		—	
Pitaya						
Red pitaya (*Hylocereus costaricensis*)	85.2	256	rutin	1.2	—	
Yellow pitaya (*Selenicerus megalanthus*)	81.1	456	—		—	
Plum						
European plum (*Prunus domestica*)						
raw	85.3	3809	(+)-catechin	19.9	caffeic acid	5.5
			procyanidin B1	17.9	chlorogenic acid	30.8
					cryptochlorogenic acid	4.0
					neochlogenic acid	223.0
dried	39.7	3141	—		caffeic acid	11.7
					chlorogenic acid	35.2
					cryptochlorogenic acid	142.7
					neochlogenic acid	369.1
Japanese plum (*Prunus salicina*)	91.1	1486	(+)-catechin	20.9	chlorogenic acid	3.0
			(-)-epicatechin	7.1	cryptochlorogenic acid	6.1
			procyanidin B1	25.7	neochlogenic acid	23.8
			procyanidin B2	12.7		
			procyanidin C1	2.0		
Pomegranate (*Punica granatum*)	85.3	1447	cyanidin-3-glucoside	2.5	—	
			cyanidin-3,5-diglucoside	2.0		
			pelargonidin-3-glucoside	0.2		
			cyanidins [b]	23.4−40.9		
			delphinidins [b]	6.3−13.2		
			total anthocyanins [c]	7.6−13.5		
			total anthocyanidins [d]	30.4−55.3		
			quercetin glycosides [e]	0.9		

Table 1-6. (Continued).

Food (*scientific name*)	Water content (%)	Total polyphenol content [a] (μmol/100 g)	Polyphenol content (μmol/100 g fresh edible part): flavonoids		simple polyphenols	
Quince	79.1	3087	—		chlorogenic acid	91.1
(*Cydonia oblonga*)					neochlorogenic acid	76.7
Chinese quince	85.7	2702	procyanidin B2	4.9	chlorogenic acid	53.0
(*Chaenomeles sinensis*)					neochlorogenic acid	75.6
Rasberry	87.5	2047	(-)-epicatechin	8.5	—	
(*Rubus idaeus*)			cyanidin-3-rutinoside	0.3−1.6		
			cyanidin-3-glucoside	0.6−1.2		
			total anthocyanins [c]	41.8		
			total anthocyanidins [d]	116.0−141.0		
			quercetin glycosides [e]	1.6		
Satsuma mandarin						
(*Citrus unshiu*)						
segments						
early ripening type	90.2	956	hesperidin	233.9	neochlorogenic acid	4.9
			narirutin	85.9		
			didmyn	10.0		
			rutin	3.1		
normal ripening type	88.7	972	hesperidin	291.1	neochlorogenic acid	4.6
			narirutin	283.2		
			didmyn	26.1		
			rutin	3.7		
juice sacs						
early ripening type	90.8	712	hesperidin	112.0	neochlorogenic acid	5.8
			narirutin	58.6		
			rutin	3.7		
normal ripening type	89.8	962	hesperidin	139.8	neochlorogenic acid	5.0
			narirutin	95.6		
			didmyn	5.2		
			rutin	3.3		
Strawberry	88.3−92.0	987	(+)-catechin	8.6	—	
(*Fragaria grandiflora*			pelargonidin-3-glucoside	1.7−4.1		
=*Fragaria* × *ananassa*)			cyanidins [b]	8.9		
			pelargonidins [b]	23.3		
			total anthocyanins [c]	2.9−6.3		
			total anthocyanidins [d]	45.6		
			quercetin glycosides [e]	0.4−3.4		
Sudachi (*Citrus sudachi*)						
fruit juice	—	313	narirutin	107.2	—	
			hesperidin	21.1		
			naringin	17.2		
			eriocitrin	9.5		
			neohesperidin	9.0		

Table 1–7. (Continued).

Food (*scientific name*)	Water content (%)	Total polyphenol content [a] (μmol/100 g)	Polyphenol content (μmol/100 g fresh edible part) flavonoids		simple polyphenols	
Watermelon (*Citrullus vulgaris*)	91.7	181	—		—	
White sapote (*Achras zapota*)	81.8	980	procyanidin B2	87.1	caffeic acid	8.2
			procyanidin C1	25.8		
Yuzu (*Citrus junos*)						
fruit juice						
early ripening type	—	unexamined	narirutin	44.6	cryptochlorogenic acid	1.1
			hesperidin	18.8		
			naringin	10.4		
			neohesperidin	4.8		
normal ripening type	—	unexamined	narirutin	29.7	cryptochlorogenic acid	0.9
			hesperidin	14.9		
			naringin	7.3		
			neohesperidin	4.1		

[a] Total polyphenol contents were expressed as mmol/100 g gallic acid equivalents by the Folin-Ciocalteu method.
[b] Contents of these aglycons were determined by hydrolysis.
[c] Contents were determined using cyanidin-3-glucoside.
[d] Contents were determined using cyanidin.
[e] Contents of these glycosides were determined using aglycons.

and Satsuma mandarins also had large amounts of chlorogenic acids. The efficiency of mass spectrometric techniques had been described in a review on phenolic acids in foods (Robins, 2003). In the present study, we used LC/MS to identify the simple polyphenols.

Several analytical studies of anthocyanins in blueberries have been reported (Dugo *et al.,* 2001; Faria *et al.,* 2005). We identified each anthocyanin in blueberries using the HPLC conditions reported previously by comparison with standard anthocyanin compounds. Other red fruits such as acerolas, pomegranates and strawberries also have anthocyanins. Anthocyanidin peaks identified by hydrolysis were presented such as cyanidins and delphinidins. But anthocyanin peaks not in agreement with standard chemicals were quantified with cyaniding-3-glucoside and were presented as total anthocyanins. Anthocyanidin peaks obtained by hydrolysis of these unknown anthocyanins were similarly quantified with cyanidin and were presented as total anthocyanidins. Although red pitaya has a bright red color, no anthocyanidins were detected. Color pigments of this fruits were expected to be betacyanins not anthocyanins (Wybraniec and Mizrahi, 2002). We could not quantitatively analyze the betacyanins because the standard chemicals were not available.

Many flavonoids have been isolated and identified from various plants including fruits. Flavonoids have been the subject of considerable scientific and therapeutic researches (Havsteen, 2002). We identified each flavonoid glycoside using three HPLC conditions. Most flavonoids occur in glycoside forms. To identify the aglycon of each flavonoid, we performed hydrolysis or the LC/MS/MS analysis. The flavonoid glycoside peaks not in agreement with standard chemicals but with aglycons identifiable by hydrolysis were quantified their aglycons and were presented such as quercetin glycosides. Consequently, as shown in Table 1, quercetin glycosides have been detected in various fruits. The flavanones narirutin and hesperidin were detected only in citrus fruits such as Satsuma mandarins and sudachi, while eriocitrin was detected in lemons and limes. These results were in agreement with those reported by Sakakibara *et al.* (2003).

In the present study, we analyzed polyphenols in fruits consumed in Japan. Although there have been many reports on polyphenols, there are few on the sample preparation and analysis under systematic conditions. The present findings may be helpful for understanding the physiological properties of polyphenols in fruits. In this study, however, several components reported previously were not detected, or the values obtained were different from the reported values. This is considered to be due to the difference between the quality or harvest season of fruits used in this study. Analysis of only the edible parts of fruits in this study may also be a reason. Perishable foods such as fruits and vegetables are different in their seasonal nature. Furthermore flavonoid content is known to be highly dependent on the cultivar and growing and processing conditions (Harnly *et al.,* 2006). In Table 1, the results analyzed three times from different fruits (same kind) were also shown. Thus, it is considered that the results with the wide range are due to the difference of samples not inaccuracy of the analytical methods.

Some of the present data are available through the web page (http: //www.nihn.go.jp/FFF/). A more complete database of functional food factors will be useful for further analyses of their effects on human health. Furthermore, the present findings should prove valuable for elucidating the roles that polyphenols in fruits may play in promoting health in Japan.

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