Quantitative Analysis of Flavan-3-ols in Spanish Foodstuffs and Beverages

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An HPLC method, using detection after postcolumn derivatization with *p*-dimethylaminocynnamaldehyde (DMACA), was developed for the quantitative analysis of individual flavanols in food. This method was applied to flavanol determination in 56 different kinds of Spanish food products, including fruit, vegetables, legumes, beverages (cider, coffee, beer, tea, and wine), and chocolate. The determined compounds corresponded to the catechins and proanthocyanidin dimers and trimers usually present in food and, therefore, they were representative of the flavanols of low degree of polymerization consumed with the diet. The data generated could be used for calculation of the dietary intake of either individual or total flavanols, which would allow the further establishment of epidemiological correlations with the incidence of chronic diseases. Similar flavanol profiles were found in the different samples of a similar type of product, even though important variations could exist in the concentrations of total and individual flavanols among them. This was attributed to factors such as sample origin, stage of ripeness, post-harvesting conservation, and processing. Total flavanol contents varied from nondetectable in most of the vegetables to 184 mg/100 g found in a sample of broad bean. Substantial amounts were also found in some fruits, such as plum and apple, as well as in tea and red wine. Epicatechin was the most abundant flavanol, followed by catechin and procyanidin B2. In general, catechins were found in all the flavanol-containing products, but the presence of gallocatechins was only relevant in pomegranate, broad bean, lentil, grape, wine, beer, and tea, and most of the berries. Galloyled flavanols were only detected in strawberry, medlar, grape, and tea.

Keywords: *Procyanidin; foodstuff; beverage; fruit; HPLC*

INTRODUCTION

It has been suggested that flavan-3-ols (catechins and proanthocyanidins) play an important role in the prevention of some pathologies, such as cardiovascular diseases and certain forms of cancer (Santos-Buelga and Scalbert, 2000). In in vitro assays and animal systems they have been shown to possess distinct biological activities: antioxidant and free radical scavengers (Scott et al., 1993; Terao et al., 1994; Teissedre et al., 1996; Plumb et al., 1998; Saint-Cricq de Gaulejac et al., 1999; Benzie and Szeto, 1999), inhibition of tumor initiation and promotion in skin and other organs (Gali et al., 1994; Valcic et al., 1996), antibacterial and angioprotective properties (Vennat et al., 1988), and inhibition of platelet aggregation (Chang and Hsu, 1989). However, very little is still known about the actual effects of the dietary flavanols in the human body. The elucidation of such effects requires not only having sufficient information on their activity, but also on their occurrence in the diet, so that adequate epidemiological correlations with the incidence of chronic diseases can be established.

Different proanthocyanidins may have different biological effects and, thus, it is important to know not only the total amount of flavanols in the diet, but also the content of individual compounds. However, our knowledge of their occurrence in food is still fairly poor, because of the lack of sufficiently selective methods for their analysis. The different methods proposed for the quantification of proanthocyanidins differ in their basic principles and specificity and, taking into account the diversity of compounds that exists and their distinct reactivities, none of the methods can be considered totally satisfactory. Reversed-phase HPLC with UV detection, first applied by Lea et al. (1979) to the analysis of flavanols, has become the choice technique for the analysis of catechins and proanthocyanidin dimers and trimers. However, in food extracts, flavanols are usually present as complex mixtures and accompanied by other UV-absorbing phenolics, sometimes in larger amounts or with higher UV extinctions than the flavanols, thus making their chromatographic separation and detection difficult. Furthermore, as no standards are commercially available, the accurate identification of peaks in the chromatograms often remains difficult, and quantitative data usually have to be expressed in catechin equivalents. For these reasons, although relevant progress has recently been made in the knowledge of the occurrence of other flavonoids in food, scarce information is yet available on flavanols, especially with regard to the content of individual flavanols, as determined by HPLC. Only some products such as tea (e.g., Dalluge et al., 1998; Lin et al., 1998) and red wine (e.g., Bourzeix et al., 1986; Ricardo da Silva et al., 1992) have been studied extensively, and some quantitative data have also been contributed for grapes (Bourzeix et al., 1986; Ricardo da Silva et al., 1992; Spanos and Wrolstad, 1992), apples (Mayr et al.,

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10.1021/jf000549h CCC: $19.00 © 2000 American Chemical Society
Published on Web 10/07/2000

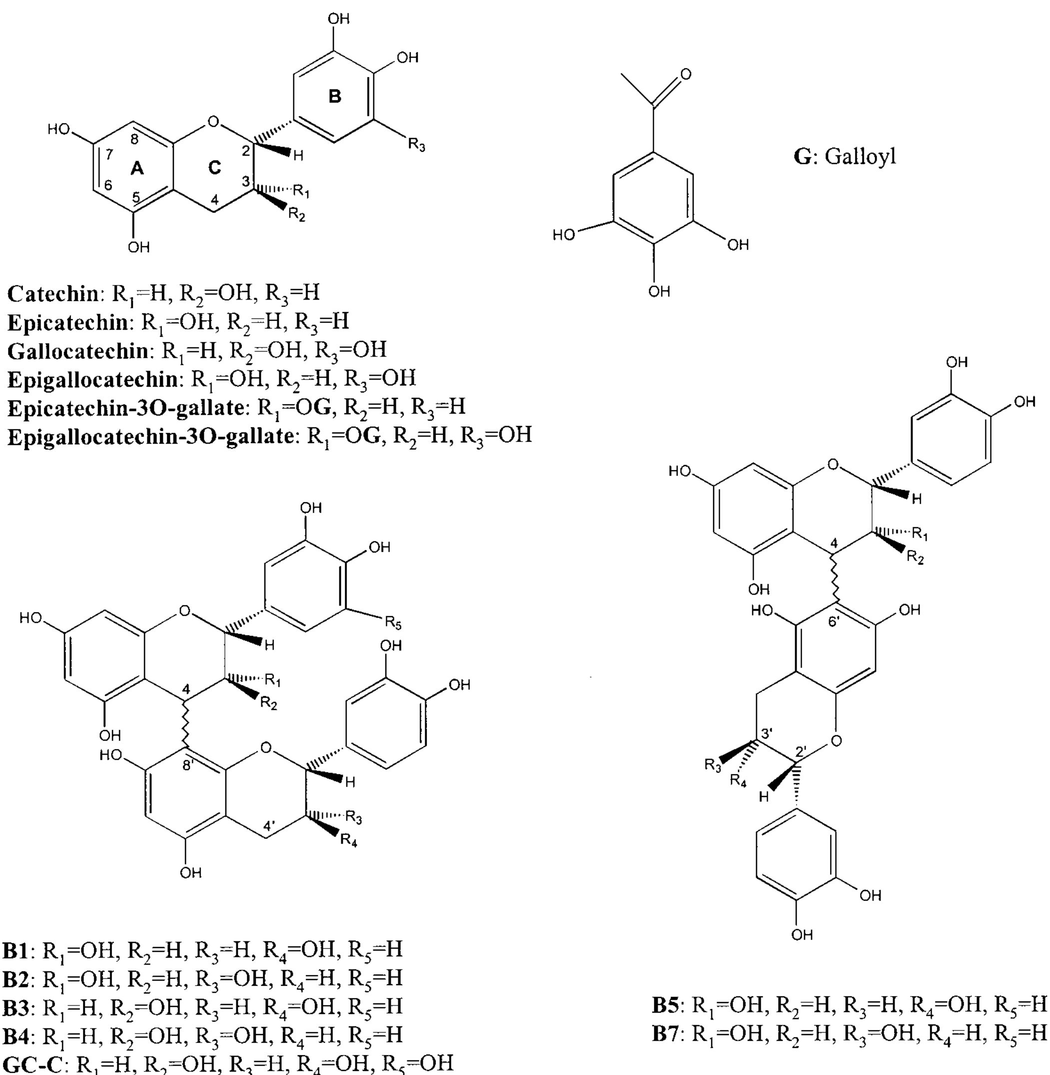

Figure 1. Structures of catechins and proanthocyanidins.

1995; Spanos and Wrolstad, 1992; Suarez-Valles et al., 1994), pears (Amiot et al., 1995; Spanos and Wrolstad, 1992), beer (McMurrough and Baert, 1994), and berries (Heinonen et al., 1998). The information available in this respect has recently been reviewed by Santos-Buelga and Scalbert (2000). The lack of reliable quantitative data concerning the distribution and content of flavanols in food products makes it impossible to accurately evaluate their dietary intake.

In this work, a methodology for the quantitative analysis of flavan-3-ols has been optimized, using HPLC separation and double on-line detection by diode array spectroscopy after a chemical reaction with *p*-dimethylaminocynnamaldehyde (DMACA), which provides a selective detection of these compounds (Treutter, 1989; Treutter et al., 1994a,b; de Pascual-Teresa et al., 1998b). The method has been calibrated for the quantitative analysis of flavanols with standards previously obtained in our laboratory from grape seeds, unripe almond fruits, and pomegranate peel (Escribano-Bailón et al., 1992; de Pascual-Teresa et al., 1998a; de Pascual-Teresa et al., 2000). By applying this method, data on the content and distribution of flavanols of a low degree of polymerization (monomers, dimers, and trimers), likely to be absorbed through the gut barrier, were obtained in a comprehensive range of Spanish foodstuffs and beverages.

MATERIALS AND METHODS

Preparation of Flavanol Standards. Different flavan-3-ols were isolated from grape seeds, pomegranate peel, and fresh almond fruit. The starting materials were repeatedly extracted with methanol, and the extracts obtained were fractionated on a Sephadex LH-20 column (45 × 5 cm i.d.) using ethanol as a solvent; (gallo)catechins and proanthocyanidins were further separated from the Sephadex fractions by semipreparative HPLC, as described in de Pascual-Teresa et al. (1998a). Thus, fifteen compounds (six monomers, seven dimers, and two trimers) were isolated; their purity was checked by HPLC−DAD and LC−MS (de Pascual-Teresa et al., 2000). The compounds were epicatechin (EC), catechin (C), epigallocatechin (EGC), gallocatechin (GC), epicatechin-3,*O*-gallate (ECG), epigallocatechin-3,*O*-gallate (EGCG), procyanidin dimers B1, B2, B3, B4, B5, and B7, prodelphinidin GC-(4,8)-C (GC-C), and procyanidin trimers C1 and EC-(4,8)-EC-(4,8)-C (EEC) (Figure 1).

Samples. Samples corresponding to 56 different Spanish foodstuffs and beverages were collected from local markets: *fruit and berries*, including apple (four different varieties), apricot, strawberry-tree fruit, avocado, banana, blackberry, blueberry, cherry, chestnut, custard apple, early fig, grape (red and white), kiwi, medlar, peach, pear (two different varieties), persimmon, pineapple, plum, pomegranate, quince, raspberry, red currant, and strawberry; *vegetables*, including aubergine, broad bean, carrot, courgette, French bean, lettuce, onion, pea, pepper (red and green), and tomato; *pulses*, including chickpea, lentil, pinto bean, and white bean; *beverages*, including soluble

Table 1. Average Concentrations of Selected Flavanols in Spanish Foodstuffs (expressed as mg/100 g fresh weight) and Beverages (mg/100 mL)

product	GC[a]	GC−C	B3	C	B1	EEC	EGC	B4	B2	EC	EGCG	B7	C1	ECG	B5	total
								Fruits and Berries								
Apple, Golden			0.10 (163%)[b]	0.15 (160%)	0.52 (127%)	0.43 (158%)			3.21 (60%)	2.00 (58%)		0.57 (88%)	2.45 (53%)	0.19 (173%)	0.18 (173%)	9.83 (64%)
Apple, Granny Smith			0.16 (28%)	0.60 (10%)	1.68 (10%)	0.86 (26%)			4.13 (78%)	2.66 (79%)		3.25 (13%)	6.21 (16%)		0.49 (57%)	20.04 (16%)
Apple Renette			0.31 (18%)	1.38 (9%)	3.66 (3%)	1.73 (14%)			11.47 (11%)	6.89 (12%)		5.75 (6%)	9.72 (7%)		1.97 (6%)	42.89 (6%)
Apple, Red Delicious			0.35 (12%)	1.57 (14%)	3.37 (5%)	2.44 (13%)			7.88 (18%)	6.37 (10%)		5.49 (8%)	9.71 (10%)		1.23 (9%)	38.40 (6%)
Apricot			0.05 (51%)	0.31 (47%)	0.09 (96%)	0.01 (108%)	NQ[c]		NQ	0.02 (172%)		0.01 (182%)	NQ			0.50 (38%)
Avocado									0.02 (97%)	0.08 (77%)						0,10 (42,5%)
Banana		0.10 (22%)					0.01 (92%)			0.03 (116%)						0.14 (48%)
Blackberry		0.07 (88%)	0.33 (34%)	0.79 (32%)	0.23 (106%)	0.06 (173%)	0.36 (104%)		0.83 (30%)	2.67 (11%)						5.33 (15%)
Blueberry	0.59 (58%)		0.08 (8%)	0.81 (57%)	0.75 (65%)		0.37 (57%)	0.37 (52%)	0.19 (44%)	0.30 (53%)		0.27 (56%)	0.73 (81%)			5.27 (58%)
Cherry	NQ		0.08 (93%)	0.61 (3%)	0.23 (85%)		0.11 (20%)	0.18 (21%)	2.10 (14%)	5.45 (15%)		1.01 (55%)	1.85 (31%)	0.20 (88%)	0.20 (87%)	12.03 (17%)
Chestnut	0.01 (173%)		0.01 (157%)	0.01 (183%)						NQ			0.02 (175%)			0.05 (173%)
Custard apple			0.31 (61%)	0.58 (38%)	1.27 (33%)	0.97 (28%)		2.48 (32%)	7.01 (36%)	5.63 (35%)		2.31 (96%)	3.52 (38%)	0.04 (174%)	0.82 (93%)	24.92 (25%)
Early fig			0.03 (42%)	0.07 (24%)						0.05 (36%)						0.15 (32%)
Grape, red			0.12 (56%)	0.82 (52%)	0.43 (33%)		0.08 (67%)	0.33 (19%)	0.36 (3%)	0.70 (4%)			0.38 (82%)	0.17 (105%)		3.39 (35%)
Grape, white	0.03 (88%)	0.12 (89%)	0.32 (141%)	0.39 (41%)	0.47 (41%)	0.13 (91%)	0.04 (89%)	0.06 (87%)	0.04 (36%)	0.07 (16%)			0.07 (88%)			1.75 (43%)
Kiwi									0.14 (10%)	0.27 (78%)			0.11 (58%)			0.52 (49%)
Medlar			0.05 (71%)	0.02 (94%)	0.14 (9%)	0.11 (94%)	0.01 (114%)	0.13 (23%)	0.78 (5%)	0.53 (10%)		0.17 (111%)	0.52 (37%)	0.23 (40%)	0.03 (107%)	2.70 (8%)
Peach			0.07 (87%)	0.53 (151%)	0.71 (75%)	0.34 (90%)		0.13 (100%)	2.32 (51%)	0.65 (156%)		1.30 (142%)	2.53 (171%)	0.01 (121%)	0.04 (176%)	8.62 (83%)
Pear, Blanquilla			0.01 (177%)	0.01 (171%)		0.03 (173%)			0.73 (123%)	0.91 (127%)			0.40 (134%)		0.01 (167%)	2.10 (128%)
Pear, Conferencia					0.02 (92%)				0.59 (28%)	1.02 (4%)		0.09 (103%)	0.44 (13%)			2.17 (8%)
Persimmon	0.17 (8%)	0.30 (22%)	0.01 (94%)	0.63 (37%)	0.13 (93%)	0.04 (87%)										1.27 (30%)
Pineapple										NQ						NQ
Plum			1.00 (43%)	5.21 (56%)	8.84 (53%)	7.73 (36%)		1.02 (67%)	5.20 (61%)	4.35 (82%)		4.69 (43%)	10.01 (48%)		1.59 (69%)	49.65 (52%)
Pomegranate	0.17 (52%)		0.16 (55%)	0.40 (21%)	0.13 (87%)		0.16 (72%)			0.08 (90%)						1.09 (19%)
Quince		0.08 (89%)	0.10 (60%)	0.75 (85%)	0.73 (76%)	0.28 (50%)		0.24 (43%)	1.34 (89%)	0.67 (89%)			0.94 (88%)		0.12 (97%)	5.24 (33%)
Raspberry	0.01 (143%)		0.03 (174%)	0.05 (172%)	NQ		1.11 (12%)		2.79 (24%)	1.78 (22%)		0.18 (109%)	0.30 (94%)			6.24 (28%)
Redcurrant	1.35 (25%)	1.70 (24%)	0.20 (89%)	1.33 (21%)			0.36 (94%)			0.19 (21%)						5.14 (29%)
Strawberry	0.12 (40%)		1.10 (18%)	1.57 (24%)	0.62 (73%)	0.50 (51%)	0.15 (63%)	0.13 (5%)	0.04 (39%)	0.02 (113%)				0.66 (29%)		4.91 (23%)
Strawberry tree fruit	1.60 (48%)	1.99 (24%)	1.69 (5%)	7.48 (36%)	1.72 (63%)	2.62 (70%)		0.43 (78%)	0.22 (89%))	1.11 (87%)		0.55 (87%)	1.07 (87%)			20.47 (47%)
								Vegetables								
Aubergine																
Broad bean	9.68 (15%)	23.50 (13%)	8.17 (13%)	16.23 (45%)	11.26 (7%)		17.38 (40%)	18.47 (14%)	12.08 (17%)	37.55 (27%)			0.13 (173%)			154.46 (17%)
Carrot																
Courgette																
Lettuce																
Onion																
Pea				0.01 (89%)						0.01 (91%)						0.02 (90%)
Pepper, red																
Pepper, green																
Tomato																

Table 1. (Continued)

product	GC[a]	GC−C	B3	C	B1	EC-EC−C	EGC	B4	B2	EC	EGCG	B7	C1	ECG	B5	total
Pulses																
Chickpea																
French bean																
Lentil	0.14	0.45	0.59	0.35												1.52
	(104%)	(27%)	(55%)	(4%)												(39%)
Pinto bean			0.82	5.07	1.22		0.05		0.12	0.14						7.42
			(20%)	(16%)	(89%)		(93%)		(30%)	(88%)						(22%)
White bean			0.03	0.01						0.09						0.13
			(97%)	(111%)						(94%)						(96%)
Beverages																
Cider									NQ	0.03						0.03
										(26%)						(36%)
Coffee							0.05			0.06						0.11
							(12%)			(10%)						(9%)
Soluble cacao			0.07	0.74	0.01				0.28	0.59			0.13			1.78
			(40%)	(2%)	(52%)				(10%)	(14%)			(37%)			(9%)
Tea, black	2.08	1.65	0.49	1.25	2.50		4.08	1.80	0.57	6.60	1.35	0.46	0.76	3.23	0.01	26.80
	(11%)	(8%)	(56%)	(34%)	(46%)		(7%)	(48%)	(80%)	(57%)	(87%)	(92%)	(87%)	(62%)	(167%)	(28%)
Tea, green	3.07	0.27	0.37	0.74	0.56		10.72	1.83	0.75	10.61	4.62	0.63	1.07	8.59		43.83
	(5%)	(45%)	(9%)	(13%)	(66%)		(43%)	(23%)	(72%)	(61%)	(37%)	(88%)	(92%)	(25%)		(37%)
Wine, red	0.42	0.11		1.78	2.15	NQ	0.28	0.08	0.43	1.14		0.27	0.22	NQ	NQ	2.66
	(36%)	(47%)		(51%)	(25%)		(5%)	(27%)	(138%)	(23%)		(38%)	(40%)			(39%)
Wine, rose	0.18			0.71	0.57		0.07	0.02	0.21	0.37		0.06	0.01			2.20
	(47%)			(23%)	(17%)		(19%)	(64%)	(42%)	(14%)		(38%)	(52%)			(24%)
Wine, white	0.01			0.10	0.03			NQ	0.01	0.06		NQ				0.20
	(120%)			(22%)	(51%)				(22%)	(15%)						(16%)
Beer	0.10	0.21	0.80	0.73					0.16	0.18			0.07			0.64
	(26%)	(16%)	(21%)	(15%)					(155%)	(34%)			(30%)			(31%)
Others																
Bee polen										NQ						NQ
Chocolate			0.24	1.25	0.24				1.64	2.18			1.38		0.44	7.37
			(174%)	(168%)	(173%)				(154%)	(146%)			(171%)		(173%)	(160%)
Wheat flour																

[a] Abbreviations: EC, epicatechin; C, catechin; EGC, epigallocatechin; GC, gallocatechin; ECG, epicatechin-3O-gallate; ECGC, epigallocatechin-3,O-gallate; B1, EC-(4,8)-C; B2, EC-(4,8)-EC; B3, C-(4,8)-C; B4, C-(4,8)-EC; B5, EC-(4,6)-EC; B7, EC-(4,6)-C; GC-C, GC-(4,8)-C; C1, EC-(4,8)-EC-(4,8)-EC; EEC, EC-(4,8)-EC-(4,8)-C. [b] Figures in brackets indicate the coefficient of variation ($n = 3$). [c] NQ, amount not quantifiable.

cacao, cider, coffee, beer, tea (green and black), wine (red, rosé, and white); and *others*, consisting of chocolate, wheat flour, and bee pollen. For each product three samples, which were collected at a different time or in a different grocery, were analyzed separately.

Sample Preparation. Solid samples were immediately washed, peeled (in the cases of banana, chestnut, custard apple, early fig, kiwi fruit, medlar, persimmon, pineapple, pomegranate, and quince), cut into slices (when necessary), and frozen to be further freeze-dried. Freeze-dried samples were maintained in a helium atmosphere at −30 °C until their analysis. Moisture content was determined from the weight of the samples before and after freeze-drying. For their analysis freeze-dried samples (3 g) were homogenized in cold methanol (3 × 25 mL) and centrifuged. Water was added to the supernatants, the methanol was evaporated under vacuum, and the aqueous extract obtained was made up to 10 mL with water. An aliquot was injected into the HPLC system after filtration in a 0.45-μm membrane-filter. Beverages were directly injected after filtration; carbon dioxide was eliminated under vacuum when necessary. In the cases of coffee, tea, and soluble cacao, aqueous extracts were previously prepared by percolation, infusion, or suspension in water, respectively, according to the usual way of preparation in the home. The weights of coffee, tea, and cacao used per 100 mL of water were 3 g, 2 g, and 5 g, respectively.

Analysis of Flavan-3-ols. The analysis of flavanols was carried out by HPLC in the same conditions of separation and detection described in de Pascual-Teresa et al. (1998b). Quantification of individual flavanols was performed from the areas of their peaks recorded at 640 nm (after postcolumn derivatization with DMACA) by comparison with calibration curves obtained using standard solutions of the fifteen compounds previously isolated. The volume of sample injected was that appropriate for the areas of the peaks being included in the range of concentrations used for the preparation of the calibration curves (10 to 100 μg/mL). The concentrations of flavan-3-ols in the products analyzed were expressed in mg/100 g of fresh weight in solid food and in mg/100 mL in beverages. For the calculation of the content, on a fresh weight basis, the moisture of the sample was taken into account.

RESULTS AND DISCUSSION

The HPLC technique, with detection after chemical reaction with DMACA, had been used previously for the analysis of the qualitative flavanol profiles in plant and food extracts (de Pascual-Teresa et al., 1998b). However, this highly selective detection had not been applied to the quantitative analysis, as no information existed on the responses obtained at 640 nm (A_{640}) for the distinct flavanols after their reaction with the DMACA reagent. Because no commercial flavanol standards were available, several flavanol monomers, dimers, and trimers were isolated from suitable plant sources so that their A_{640} response could be established. Fifteen compounds were thus obtained and used as standards for the calibration of the technique (see Experimental Section). Good linearity was obtained for all of the compounds in the range of concentrations tested (10 to 100 μg/mL).

Methanol was used for extraction of the food samples because it has proven to be a good solvent for the extraction of flavanols of low degree of polymerization (Kallithraka et al., 1995). To check recoveries, external

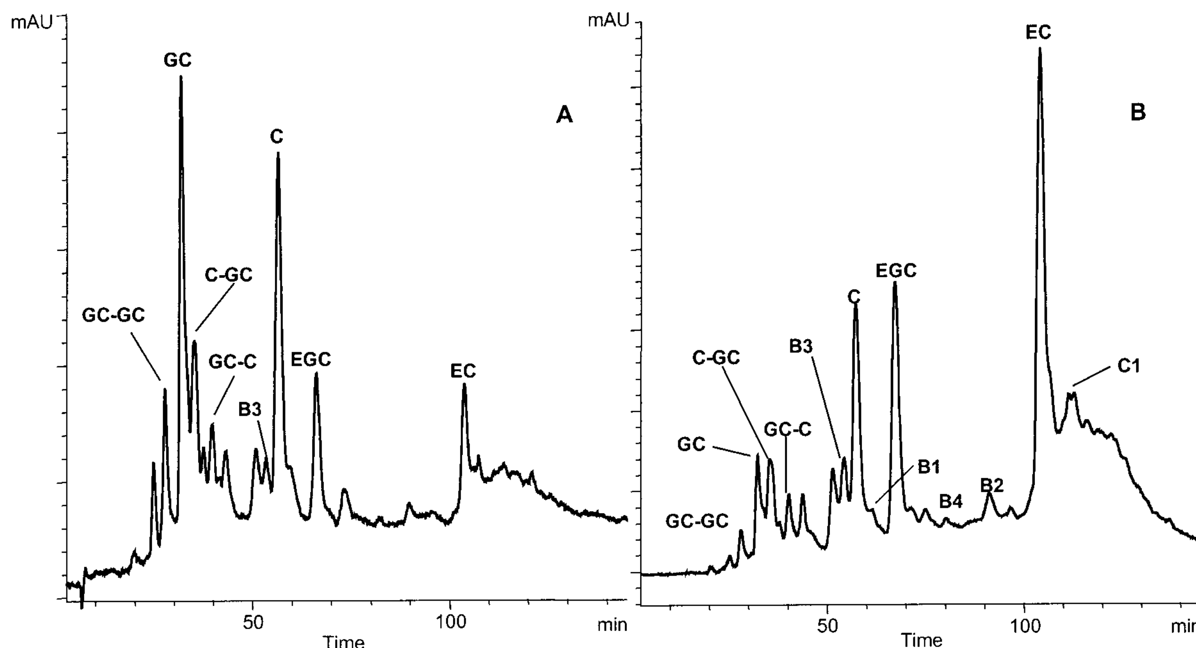

Figure 2. HPLC chromatograms recorded at 640 nm after reaction with *p*-dimethylaminocynnamaldehyde (DMACA) corresponding to samples of red currant (A) and broad bean (B). See Tables 1 and 2 for abbreviations of flavanols.

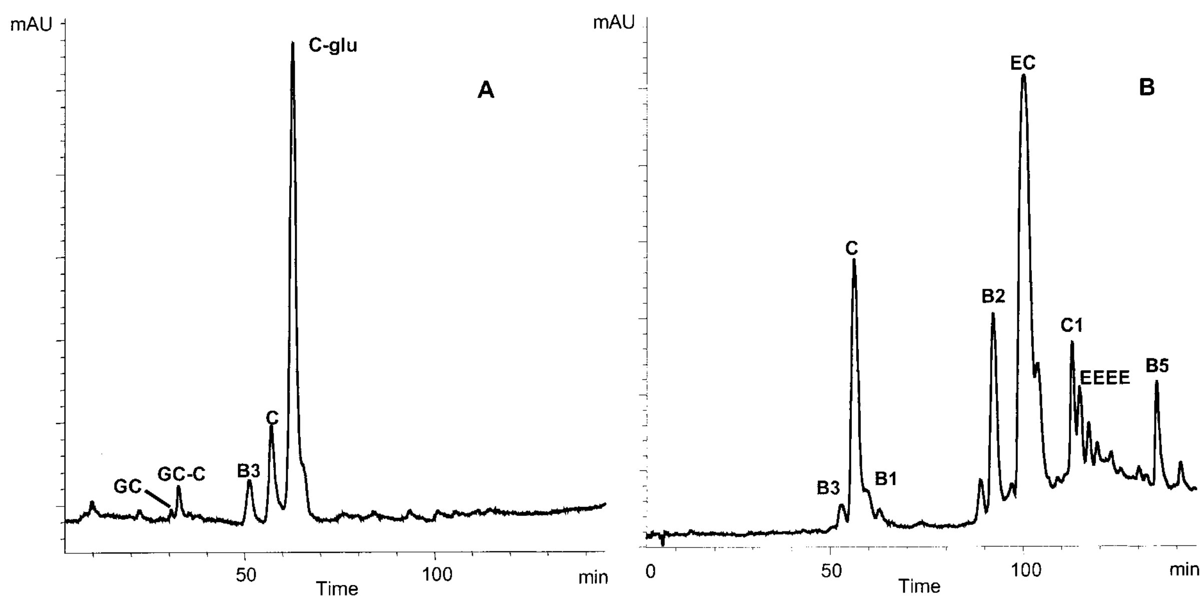

Figure 3. HPLC chromatograms recorded at 640 nm after reaction with *p*-dimethylaminocynnamaldehyde (DMACA) corresponding to samples of lentils (A) and plain chocolate (B). See Tables 1 and 2 for abbreviations of flavanols.

flavanol standards (EC, procyanidin dimer B2, and trimer C1) were incorporated into a powdered sample of freeze-dried pomegranate in the range of flavanol concentrations expected in the product (concentrations added of each compound were 0.02, 0.04, and 0.06 mg/100 g). The recoveries obtained were 85% in the case of the monomer, 107% in the case of the dimer, and 91% for the trimer, on average. The accuracy of the analytical methodology was also calculated. With this aim, samples of a solid product (pomegranate) which required extraction prior to the HPLC analysis, and of a beverage (red wine) which was directly injected into the HPLC system, were analyzed. The coefficients of variation of repeatability ($n = 10$) obtained for the concentrations of the individual flavanols determined in each product were GC (9.6%), EGC (13.1%), C (4.3%), B1 (5.4%), and B3 (7.6%), in the case of pomegranate; and GC (3.9%), EGC (7.7%), C (2.1%), EC (2.9%), B1 (1.8%), B2 (3.4%), B3 (8.8%), B7 (7.8%), EEC (11.8%), and C1 (12.6%), in that of red wine. These results were considered acceptable taking into account the variety of compounds to be analyzed and the lack of a universal solvent that could provide the complete extraction of all the flavanols (Kallithraka et al., 1995). It is also assumed that extraction may be influenced by the composition of the samples and, particularly, by their contents in fat and proteins, as further stressed for the samples of chocolate or legumes.

The method was then applied to the analysis of the individual contents of fifteen flavanols (those used for the calibration) in 56 different Spanish foodstuffs and beverages. The results obtained are shown in Table 1, in which the global content of flavanols of low degree of polymerization (monomers plus dimers plus trimers), calculated from the sum of the concentrations of the individual compounds determined, is also included.

Table 2. Flavanols Detected in Some Products that Could Not Be Quantified

sample	GC-GC[a]	C-GC	C-glu	EEEC	B6	EEEE
Apple				+		+
Apricot				+		+
Avocado						+
Beer	+	+				
Blueberry	+	+		+	+	+
Broad bean	+	+		+	+	+
Cherry						+
Chestnut	+	+				
Chocolate						+
Custard apple						+
Grape	+	+		+	+	+
Kiwi						+
Lentil	+	+	+	+		
Medlar						+
Peach						+
Pear						+
Persimmon		+		+		
Pinto bean				+	+	
Plum				+	+	+
Pomegranate	+	+		+		+
Quince				+		
Raspberry						+
Redcurrant	+	+			+	
Soluble cacao						+
Strawberry				+	+	
Strawberry tree fruit	+	+		+		
Tea	+	+				
Wine	+	+		+	+	+

[a] Abbreviations: GC-GC, GC-(4,8)-GC; C-GC, C-(4,8)-GC; C-glu, C-3-glucose; EEEC, EC-(4,8)-EC-(4,8)-EC-(4,8)-C; B6, C-(4,6)-C; EEEE, EC-(4,8)-EC-(4,8)-EC-(4,8)-EC

Figures included in the table correspond to average contents calculated from three analyses made of three different samples collected at different times or places; coefficients of variation among the contents determined in the different samples of the same product are indicated in brackets in the table. Among the foods analyzed, the highest flavanol contents were found in broad beans (average concentration of 154.5 mg total flavanols/100 g fresh weight) and some fruits such as plum, apple, custard apple, strawberry-tree fruit, and cherry, all with concentrations of total flavanol ranging from 10 mg to 50 mg/100 g fresh weight. By contrast, hardly any flavanols were found in vegetables. Among the beverages, the highest flavanol levels were determined in green and black teas (43.8 and 26.8 mg/100 mL of infusion, respectively). With regard to individual compounds, epicatechin was the flavanol most usually found in the samples analyzed, followed by catechin and procyanidin B2. In general, catechins (C and EC) were present in all the samples containing flavanols, while gallocatechins (GC and EGC) were detected in some products, including pomegranate, broad bean, lentil, grape, cherry, chestnut, persimmon, wine, beer, coffee, and tea, and most of the berries. However, gallocatechin contents were only relevant in blueberry, raspberry, pomegranate, red currant, broad bean, lentil, and green and black tea, where they represented more than 30% of the total contents of the monomers. Galloyled flavanols (ECG and EGCG) were still less common and their presence was important only in the samples of green tea, where they represented 34% of the total monomers, and samples of medlar (30%), strawberry (26%), black tea (25%),and grape (9%).

The concentrations of flavanols determined in the analyzed samples cannot be adequately compared with previous results, because the literature contains very few references to data that were obtained by a sufficiently selective technique (such as HPLC). The flavanol contents found in the samples of berries (blackberry, blueberry, raspberry, strawberry, and sweet cherry) are in the same concentration range as those determined by Heinonen et al. (1998) in the same kind of products. Similar concentrations were also found in the cases of tea (Lin et al., 1998), apple (Mayr et al., 1995; Suarez-Valles et al., 1994), pear (Amiot et al., 1995), and beer (McMurrough and Baert, 1994). However, higher flavanol contents were found by Bourzeix et al. (1986) and Ricardo da Silva et al. (1992) in grapes and red wines, which may be attributed to differences in the origin of the products (cultivation practices, variety, winemaking techniques, etc.).

The determined compounds corresponded to the catechins and proanthocyanidin dimers and trimers usually present in food and, therefore, they were representative of the flavanols of a low degree of polymerization that are consumed with the diet. Nevertheless, it is obvious that in most of the samples the flavanol content is actually higher because other proanthocyanidins may be present, which could not be quantified because of the lack of reference substances. In Table 2 other flavanols detected in some products and that were not quantified are indicated. The occurrence of nonquantified flavanols was particularly relevant in the samples of broad bean and red currant, whose chromatograms (Figure 2) showed some peaks at short retention times corresponding to (epi)gallocatechin-based proanthocyanidins that, according to their areas, should make an important contribution to the total content of flavanol oligomers in these products. Another product that showed a high content of a nonquantified flavanol was lentils, in whose chromatograms a notable peak was observed (Figure 3A). This compound was isolated and submitted to additional assays (MS, NMR, and selective cleavages) which allowed us to identify it as catechin-3,*O*-glucoside (de Pascual-Teresa and Treutter, unpublished results).

It must be emphasized that similar flavanol profiles were observed in all the different samples of the same food, indicating that the qualitative composition can be a characteristic of the product. However, important differences may exist regarding the flavanol concentrations, which may be influenced by several factors, such as origin of the sample, variety, stage of ripeness, postharvesting conservation, and processing. These differences were much more important in processed products such as tea, wine, and chocolate. In the particular case of the latter, the three samples analyzed presented identical qualitative flavanol composition, characterized by the presence of EC and C, procyanidin dimers B1, B2, B3, and B5, and the trimer C1 (Figure 3B); however, the global content of flavanols varied from 0.5 mg/100 g in milk chocolates to 20.9 mg/100 g in plain chocolates. These differences could be attributed not only to their different cacao contents (20% to 60%), but also to the presence of other ingredients, such as milk proteins or fat, which may have affected the extraction of flavanols, possibly by binding to them or making them less accessible to the solvent. Similarly, the extraction of flavanols might also be impeded in other protein-containing products, such as legumes.

In summary, the method developed is a useful tool for the quantitative analysis of flavanols of low degree of polymerization, which are more likely to be absorbed through the gut barrier. The method has been applied to their determination in foodstuffs and beverages, which allowed the obtainment of data on the individual contents of the most usual flavanol monomers, dimers, and trimers in a comprehensive range of food products,

including the products of plant origin, most usually consumed in the Spanish diet. The data contributed allow, for the first time, the formation of an idea of the levels of flavanols in different food products, as well as of their qualitative and quantitative contribution to the dietary flavanol intake. Nevertheless, taking into account the variations that may exist in the flavanol levels among different samples of the same product, the number of samples analyzed should be increased, so that more reliable data on the average flavanol concentrations in different foodstuffs can be obtained, which can be of further use in epidemiological studies.

ACKNOWLEDGMENT

Thanks to Mr. G. Jenkins for revision of the English version of the manuscript.

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Received for review May 1, 2000. Revised manuscript received August 7, 2000. Accepted August 8, 2000. Thanks to the European Union (project FAIR-CT-95-0653), the Spanish Comisión Interministerial de Ciencia y Tecnología (ALI96-0861-CE), and Junta de Castilla y León (SA71/98) for financial support.

JF000549H