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The Antioxidant Activity of Regularly Consumed Fruit and Vegetables Reflects their Phenolic and Vitamin C Composition

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Recent studies are emphasising the importance and putative modes of action of specific flavonoids as bioactive components of the diet in *in vivo* and *in vitro* models. Thus, it is important to have a clear idea of the major phenolic families of which fruit and vegetables are comprised and the levels contained therein. Regularly consumed fruit and vegetables of mixed varieties available on the UK market were analysed for the composition of the major individual phenolic components. The total phenolic content (applying the Folin assay) and the vitamin C levels were also determined. The antioxidant capacities of aqueous/methanolic extracts were comparatively assessed using the TEAC (Trolox Equivalent Antioxidant Capacity), the FRAP (Ferric Reducing Ability of Plasma) and ORAC (Oxygen Radical Absorbance Capacity) assays, which comprise contributions from polyphenols, simple phenols and the ascorbate component. The results were calculated in terms of 100 g fresh weight (FW) uncooked portion sizes. Fruit and vegetables rich in anthocyanins (e.g. strawberry, raspberry and red plum) demonstrated the highest antioxidant activities, followed by those rich in flavanones (e.g. orange and grapefruit) and flavonols (e.g. onion, leek, spinach and green cabbage), while the hydroxycinnamate-rich fruit (e.g. apple, tomato, pear and peach) consistently elicited the lower antioxidant activities. The TEAC, FRAP and ORAC values for each extract were relatively similar and well-correlated with the total phenolic and vitamin C contents. The antioxidant activities (TEAC) in terms of 100 g FW uncooked portion size were in the order: strawberry $\geqslant$ raspberry = red plum $\geqslant$ red cabbage $\ggg$ grapefruit = orange > spinach > broccoli > green grape $\cong$ onion > green cabbage > pea > apple > cauliflower $\cong$ pear > tomato $\cong$ peach=leek > banana $\cong$ lettuce.

Keywords: Flavonoid analysis; Antioxidant activity; TEAC; HPLC analysis; Anthocyanin; Flavanol; Hydroxycinnamate; Vitamin C

INTRODUCTION

Flavonoids are important constituents of fruit, vegetables and beverages which contain a wide variety of phenolics in differing amounts.[1–6] These naturally occurring phenolic compounds can be categorised into five major groups: flavonols, anthocyanins, hydroxycinnamates, flavanones, flavan-3-ols and the related oligomeric procyanidins.[7] An increasing number of studies suggest that consumption of fruit and vegetables can reduce the risk of both cancer and cardiovascular diseases, in which components such as vitamin C, vitamin E, flavonoids and carotenoids may play a protective role.[8–12] Therefore, much attention is currently being paid to the possible health benefits of dietary flavonoids.[13–18] Flavonoids, as well as flavonoid-rich foods and beverages, show a wide range of antioxidant activities *in vitro*.[19–24] Although

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it cannot be implied that all the potential beneficial effects of phenolics are related to their antioxidant activity, increasing evidence for the role of oxidative damage in disease pathology suggests that antioxidant properties are likely to be a contributing factor to the effects of flavonoids *in vivo*.[25] Furthermore, increasing evidence show that flavonoids can be absorbed into the human body in amounts that are, in principle, sufficient to exert antioxidant or other biological activities *in vivo*.[26–32] Also, unabsorbed flavonoids may elicit protective effects in the gastrointestinal tract, especially the stomach and colon.[33]

Epidemiological studies, which analyse the health implications of dietary flavonoid intake on risk of cardiovascular diseases, cancers and other pathological conditions, are often based on estimates of dietary intake in a sample population and on available databases of levels of the compounds of interest in commonly consumed foods. Therefore, the availability of appropriate food composition data is crucial for estimating dietary intake of flavonoids in relation to health benefits. Several limitations exist in the generation of reliable estimates of flavonoids intake. In particular, there is little consensus on the most appropriate analytical techniques for the determination of flavonoids in food and consequently for the construction of generally accepted food composition data. Furthermore, the flavonoid content of fruit, vegetables and beverages differ with variety and horticultural practices. Therefore it is important to have access to extensive national databases of the flavonoid contents of the varieties of the fruit and vegetables most commonly grown and consumed in the country of interest, in order to obtain consistent and reliable data on the intake of flavonoids in the population.

In this research, a rapid method for the simultaneous HPLC characterisation and quantification of the major hydroxycinnamate, flavanone, flavonol and anthocyanin components of 20 fruits and vegetables is described, providing the basis for a phenolic composition database for fruit and vegetables regularly consumed in the United Kingdom. Furthermore, their vitamin C and total phenolic contents are reported and the relationship between the phenolic composition and antioxidant potential *in vitro* is investigated, in order to identify the major contributors to the antioxidant activities of the flavonoid-rich dietary components. The results are presented and discussed in relation to the available literature data.

MATERIALS AND METHODS

Materials

All chemicals used were of analytical grade purity and obtained from Sigma-Aldrich (Poole, Dorset, UK or Zwijndrecht, The Netherlands). Trolox®, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, was purchased from Randox Ltd. (Antrim, UK). The ferric 2,4,6-tri(2-pyridyl)-*s*-triazine complex was obtained from Fluka (Zwijndrecht, The Netherlands). ABAP, 2,2′-azobis(2-methyl-propionamidine dihydrochloride was from ACROS (Geel, Belgium). Ultra-pure water (18.2 MΩ, ELGA Maxima, Buckinghamshire, UK) was used to prepare all solutions. The flavonoid standards were purchased from Extrasynthese (Genay, France). Solvents were HPLC grade and were purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland, UK) or Merck (Darmstadt, Germany). The columns were from Waters Corporation (Watford, Herts, UK). β-Glucosidase enzyme (salt-free, from almonds, activity: 3811 U/mg) was from ICN Biomedicals Inc. (Aurora, Ohio, USA).

Sample Preparation

Several varieties of each fruit and vegetable, depending on their availability, were purchased at local supermarkets. The number of varieties for each fruit and vegetable used is given in Table I. After washing and cutting (the peel was removed from oranges, grapefruits and bananas), equal amounts of each variety were pooled, mixed and weighed accurately to a total weight of ~700 g. For example if 10 varieties of a fruit or vegetable were available, 70 g of each were pooled to give 700 g of material in total. The pooled material was homogenised with 10% aqueous methanol in an equal w/v ratio. The homogenate was frozen and freeze-dried. The dry matter was then weighed, powdered under liquid nitrogen and stored in sealed containers at −70°C until determinations were carried out. Thus the pooled material for each fruit and vegetable, containing equal proportions of each of its specific varieties, was used to undertake HPLC analysis of flavonoid compositions, total phenolics, vitamin C and antioxidant capacity determinations.

Sample Extraction, β-glucosidase Enzyme Treatment and HPLC Analysis

Immediately prior to weighing, samples were re-freeze dried. A precisely weighed amount of freeze-dried material (between 0.2 and 0.5 g) was then incubated with 10 ml of methanol under controlled conditions at 70°C for 10 min, centrifuged at 800 *g* for 10 min and the supernatant was decanted into a round bottom flask. The extraction was repeated three times. The three extracts were combined, methanol removed by rotary evaporation under vacuum at 40°C and the extract reconstituted in 10 ml of water. Each fruit and vegetable was extracted a minimum of three times. An internal standard for

TABLE I Fruit and vegetables varieties used

Fruit/vegetable	Varieties
Apple	Australian Sundowner, Granny Smith, Royal Gala, Pink Lady, Red Delicious, Sturmer, Braeburns, Sundowners, Pacific Queen
Banana	Jamaican, Belise, Costa Rica
Orange	Salstiana, Jaffa Minneola, Navel, Organic Clementines, Clemenvilla, Organic Navelina, Navelina Premiums, Seville Marmalade
Grapefruit	Pink, Yellow, Organic Ruby, Jaffa
Red Plum	Black Star, Ciruela Santa Rosa, Raviota, June Black.
Strawberry	American Class I Driscoll, British
Raspberry	English, Spanish
Green grape	Egyptian Superior, Sugraone Italian, Sugraone Spanish, Puglia Italian
Peach	French Royal Glor, Spanish Ambra, Italian Yellow Nectarin, Italian White Nectarina, Italian Dixired
Pear	Packham, Taylors Gold, Conference, Forrel
Tomato	English Baby Sweet Heart, English Rosa on Vine, French Cherry on Vine, Belgian Sliary, French Delice, English Traditional
Onion	Spanish Mils, Dutch Organic, English White, British Shallots, English Baby, Austrian Organic
Leek	British, Kenyan Baby
Green cabbage	Italian Black, Colt, Savoy, Dutch White
Cauliflower	British
Broccoli	Spanish
Spinach	British
Lettuce	Oak Leaf, Lollo Rossa, Lollo Biondi, Iceberg, Round Head

HPLC analysis was added to each sample (2 μg/ml final concentration) which was then appropriately diluted with 20% aqueous methanol, containing 0.1% hydrochloric acid, for analysis by HPLC with diode array detection (DAD). For identification of the aglycones, thought to be present as glucosides, samples were treated with β-glucosidase. Reconstituted extract (1 ml, containing the internal standard) was added to ~1 mg of β-glucosidase enzyme (3811 IU), gently mixed and incubated at 37°C for 2 h. Once cool, the extract was filtered through 0.45 μm pore size PTFE filters (Millipore Corporation, Bedford, USA), appropriately diluted with 20% aqueous methanol with 0.1% hydrochloric acid and analysed by HPLC-DAD. The internal standard used was cinnamic acid except for the HPLC analysis of strawberry extracts, for which isorhamnetin was utilised.

The HPLC analysis was performed on a Hewlett Packard 1100 system (Palo Alto, CA, USA) consisting of an autosampler with Peltier temperature controller, a quaternary pump with degasser and a photodiode array detector. The column was a Nova-Pak C_{18}, 4.6 × 250 mm^2 with a 4 μm particle size and it was maintained at 30°C during analysis. Samples were injected by means of an auto-sampler with a 100 μl fixed loop and the volume of injection was 50 μl. Flow rate was 0.5 ml/min and the mobile phase was constituted by solvent A, 20% aqueous methanol with 0.1% hydrochloric acid, and solvent B, acetonitrile. The solvents were mixed using a linear gradient: 100% solvent A for 10 min, then 95% solvent A for 5 min, followed by 50% solvent A for 40 min and finally returned to 100% solvent A at 55 min for further 15 min. The identification and quantification of the peaks were carried out from (1) the retention times, (2) the spectra derived from photodiode array detection between 200 and 750 nm, in comparison with authentic standards, and (3) by spiking with standards of the suspected compounds.

Validation and Recovery

A series of analyses on selected freeze-dried materials (flavanone-rich orange, anthocyanin-rich strawberry, flavonol-rich onion and hydroxycinnamate-rich tomato) were analysed for reproducibility (coefficient of variation): each selected fruit and vegetable was extracted in triplicate, each extract was prepared in duplicate for HPLC analysis and each sample was injected twice. Recovery experiments on the same material were also performed by spiking with two different concentrations (low and high) of standards. The method validation (Table II) indicates good performance, with the coefficients of variation less than 12%, except for cyanidin-3-glucoside (16%). The recoveries of the added low and high concentration standards were in the range 77–110 and 72–124%, respectively.

Total Vitamin C Determination

Total vitamin C (ascorbic acid and dehydroascorbic acid) was determined on the freeze-dried material by an HPLC method.[34] Briefly, ascorbic acid was enzymically oxidised to dehydroascorbic acid, condensed with *o*-phenylenediamine to the fluorescent quinoxaline derivative and then measured by reversed phase HPLC with fluorimetric detection.

Determination of Total Phenolics

A precisely weighed amount of freeze-dried material (between 0.2 and 0.5 g) was incubated with 4 ml of

TABLE II Results of method validation

Control sample	Component	CV (%)	Recovery (%)	
			Low standard	High standard
Strawberry	Pelargonidin-3-glucoside	12	87	84
	Cyanidin-3-glucoside	16	97	82
Orange	Hesperidin	8	77	–
	Narirutin	5	103	108
	Neohesperidin	5	79	72
Onion	Quercetin-3-glucoside	4	107	102
Tomato	5′-Caffeoylquinic acid	3	110	124
	Rutin	5	83	85

70% aqueous methanol under controlled conditions at 70°C for 10 min in stoppered borosilicate tubes and centrifuged at 800 *g* for 10 min. The supernatant was decanted into a 10 ml volumetric flask and the extraction repeated twice. The extracts were combined and made up to 10 ml volume with 70% aqueous methanol. The extract, appropriately diluted with water, was then used for the determination of total polyphenols by the Folin–Ciocalteau method.[35] Quantification was achieved by comparison against a gallic acid calibration curve.

Sample Extraction for Antioxidant Activity Determination

A precisely weighed amount of freeze-dried material (~ 0.5 g) was extracted with aqueous methanol, in the ratio 0.5 g plant material: 10 ml H_2O/20 ml methanol and refluxed for 30 min as previously described.[22] The extract was allowed to cool down at room temperature and cleared by filtration. After methanol removal by rotary evaporation under vacuum, aliquots of the aqueous extract were stored at −70°C until antioxidant activity assays were performed.

TEAC (Trolox Equivalent Antioxidant Capacity)

The TEAC was determined according to the method of Re *et al.*[36] based on the oxidation of the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) by potassium persulphate to form a radical cation ($ABTS^{\cdot+}$). This intensely coloured radical cation has a maximum of absorption at 734 nm and the direct scavenging of the preformed radical by hydrogen-donating antioxidants resulted in the decrease of the absorbance at 734 nm. The absorbance at 734 nm was measured after 60 s from the start of the reaction on a Cary 50 Spectrophotometer (Varian, Victora, Australia). TEAC values were calculated from the percentage of reduction of the absorbance at 734 nm, indicating the ability of the compound to scavenge $ABTS^{\cdot+}$, in relation to that induced by a Trolox® standard solution, under the same experimental conditions, and are expressed as Trolox® equivalents.

FRAP (Ferric Reducing Ability of Plasma)

The FRAP was determined according to the method of Benzie and Strain[37] based on the reduction of a ferric 2,4,6-tripyridyl-*s*-triazine complex to the ferrous form. The ferrous form of the complex has a maximum of absorption at 593 nm. The absorption at 593 nm was measured after 7.5 min incubation using a COBAS-FARA II Centrifugal Analyser (Roche Diagnostics, Almere, The Netherlands). FRAP values were obtained by comparing the absorbance change at 593 nm in test mixtures with those containing ferrous ions in known concentration and are expressed as Fe^{2+} equivalents.

ORAC (Oxygen Radical Absorbance Capacity)

The ORAC was determined according to the method of Cao *et al.*[38] based on the oxidation of β-phycoerythrin, a fluorescent protein, by 2,2′-azobis (2-methyl-propionamidine dihydrochloride (ABAP), a peroxyl radical generator. Using a COBAS-FARA II Centrifugal Analyser, readings were taken at 2 min intervals over a 100 min period. ORAC values were calculated from the loss of fluorescence of β-phycoerythrin at different incubation time points, relative to a Trolox® standard solution in similar experimental conditions and were expressed as Trolox® equivalents.

RESULTS

The analysis of regularly consumed fruit and vegetables has been undertaken to determine their individual phenolic compositions in relation to total phenolics, vitamin C and total antioxidant activities applying TEAC, FRAP and ORAC assays.

The selected HPLC analytical approach is a method allowing the rapid and simultaneous determination of the major classes of phenolic

TABLE III Major phenolic components identified by HPLC-DAD (mean±SEM of a minimum of three samples) in red plum, strawberry and raspberry extracts

Component (mg/100 g FW)	Red plum	Strawberry	Raspberry
Cyanidin-3-glucoside	15.90±1.14	0.62±0.06	5.66±0.18
Cyanidin-3-rutinoside	3.54±0.20	–	0.82±0.02
Cyanidin-3-sophoroside*	–	–	22.42±0.60*
Pelargonidin-3-glucoside	–	11.25±0.79	–
3′-Caffeoylquinic acid*	10.69±0.07*	–	–
p-Coumaric glucose*	–	2.58±0.08*	2.87±0.17*
Cinnamoyl glucose*	–	6.09±0.18*	–
Quercetin-3-glucoside/conjugates	2.65±0.12	2.36±0.11	–
Kaempferol-3-glucoside/conjugates	1.32±0.04	0.46±0.02	–

*Identification implied from PDA spectra and literature data.

components, anthocyanins, flavanones, flavonols and hydroxycinnamates. The results divide the fruit and vegetables studied into four categories: anthocyanin-rich: strawberries, raspberries and red plums; flavanone-rich: oranges and grapefruit; flavonol-containing: onions, leeks, lettuce, broccoli, spinach, green cabbage and green grapes; hydroxycinnamate-rich: apples, pears, tomatoes and peaches.

Anthocyanin-rich Fruit

For the anthocyanin-rich fruit, the chromatographic separation achieved for a strawberry extract is shown in Fig. 1 and the levels of the identified components are presented in Table III. The major phenolic compound identified in strawberry extracts was pelargonidin-3-glucoside [$RT = 26.9$ min] (11.2 mg/100 g fresh weight (FW)). Cyanidin-3-glucoside [$RT = 24.1$ min] and cyanidin-3-sophoroside [$RT = 20.3$ min] were the major components in red plum (15.9 mg/100 g FW) and raspberry (22.4 mg/100 g FW), respectively. Red plum extracts also contained minor amounts of cyanidin-3-rutinoside [$RT = 25.4$ min] (3.5 mg/100 g FW), while raspberry (5.7 mg/100 g FW) and strawberry (0.6 mg/100 g FW) extracts had cyanidin-3-glucoside.

Flavanone-rich Fruit

The analysis of the flavanone-rich fruit, orange and grapefruit, is presented in Table IV and the chromatographic separation of orange extracts depicted in Fig. 2. The major phenolic components in orange extracts were hesperidin (hesperetin-7-rutinoside) [$RT = 36.2$ min] (98.5 mg/100 g FW) and narirutin (naringenin-7-rutinoside) [RT = 34.6 min] (16.1 mg/100 g FW), with lower components such as neohesperedin (hesperetin-7-neohesperidoside) [$RT = 37.1$ min] (10.5 mg/100 g FW) and didymin (isosakuranetin-7-rutinoside) [RT = 42.7 min] (5.8 mg/100 g FW). Naringin (naringenin-7-neohesperidoside) [$RT = 35.4$ min] (197.1 mg/100 g FW) was the most important flavanone and narirutin (29.2 mg/100 g FW) the second major identifiable component in grapefruit extracts.

TABLE IV Major phenolic components identified by HPLC-DAD (mean±SEM of a minimum of three samples) in orange and grapefruit extracts

Component (mg/100 g FW)	Orange	Grapefruit
Narirutin	16.11±0.30	29.17±0.44
Naringin	–	197.14±4.28
Hesperidin	98.51±3.30	–
Neohesperidin	10.49±0.20	–
Didymin	5.76±0.20	–
Ferullc acid	0.61±0.06	–

Flavonol-rich Fruit and Vegetables

The flavonol-containing fruit and vegetables, e.g. onion, leek, green grape, lettuce, broccoli, spinach and green cabbage, are reported in Table V, with the chromatographic separation exemplified for an onion extract in Fig. 3. Quercetin-3,4′-diglucoside [$RT = 30.1$ min] (34.5 mg/100 g FW) and quercetin-4′-glucoside [$RT = 37.2$ min] (27.5 mg/100 g FW) were the major phenolic compounds found in onion extracts, together with traces of quercetin-3-glucoside [$RT = 34.4$ min] (0.8 mg/100 g FW). Quercetin-3,4′-diglucoside and quercetin-4′-glucoside were identified on the basis of the retention time of the peaks (the more polar components, such as the diglucoside, elute earlier in this chromatographic system), the spectral characteristic and the literature reports, since authentic standards were not available. Quercetin conjugates, as identified from the spectral characteristics, were the main components in lettuce extracts (16.8 mg/100 g FW) (Table V). Also 5′-caffeoylquinic acid (chlorogenic acid, 6.7 mg/100 g FW) and anthocyanin conjugates [$RT = 25.6$ min] (2.5 mg/100 g FW), from a red variety, were found in these extracts. The main components in leek extracts were flavonol conjugates (total as quercetin-3-glucoside 91.2 mg/10 g FW) whose precise identification was not possible in this system. Flavonol and hydroxycinnamic con-

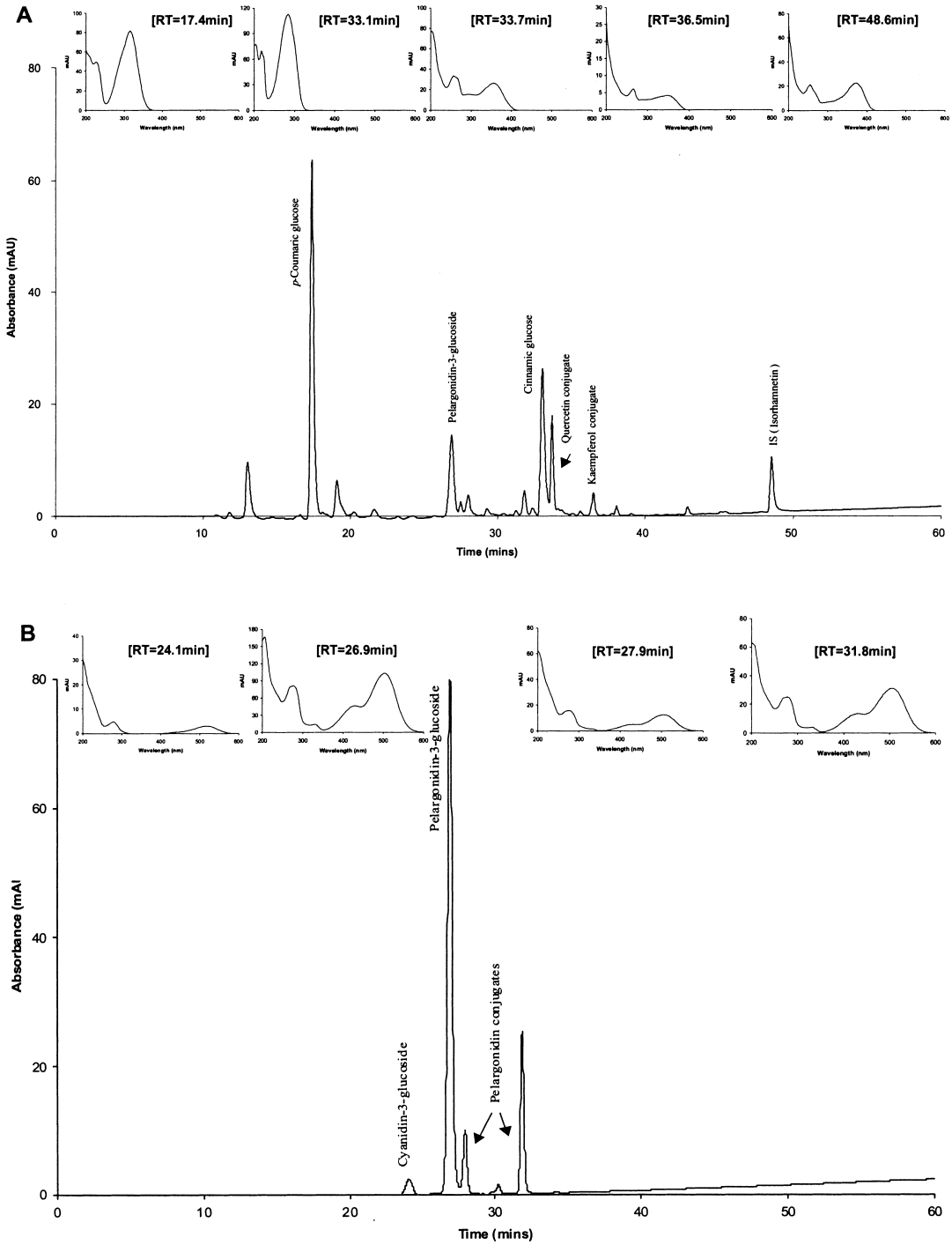

FIGURE 1 Representative HPLC-DAD (DAD monitored over the range 200–750 nm) chromatogram of an anthocyanin-rich extract, i.e. strawberry (conditions of analysis as in text). Peaks were derived at (A) 320 nm and (B) 520 nm and identified as follows: (A) *p*-coumaric glucose [$RT = 17.4$ min], cinnamic glucose [$RT = 33.1$], quercetin conjugate [$RT = 33.7$ min], kaempferol conjugate [$RT = 36.5$ min] and IS (isorhamnetin) [$RT = 48.6$ min]; (B) cyanidin-3-glucoside [$RT = 24.1$ min], pelargonidin-3-glucoside [$RT = 26.9$ min], pelargonidin conjugate [$RT = 27.9$] and pelargonidin conjugate [$RT = 31.8$ min].

jugates, as judged from the spectral characteristics, were present both in broccoli and spinach extracts but these were not specifically identified. Also, hydroxycinnamic and kaempferol conjugates were found in green cabbage. Finally, very small amounts of quercetin-3-glucoside (1.1 mg/100 g FW) and hydroxycinnamic acids, after enzymic hydrolysis, were measured in green grape extracts. Grapes are indeed characterised by cinnamoyltartrates, which the analytical system reported here could not distinguish, usually the dominating caffeoyltartaric acid also accompanied by *p*-coumaroyltartaric and feruloyltartaric acid, while the classic chlorogenic acids are absent.[3] Important components in grapes

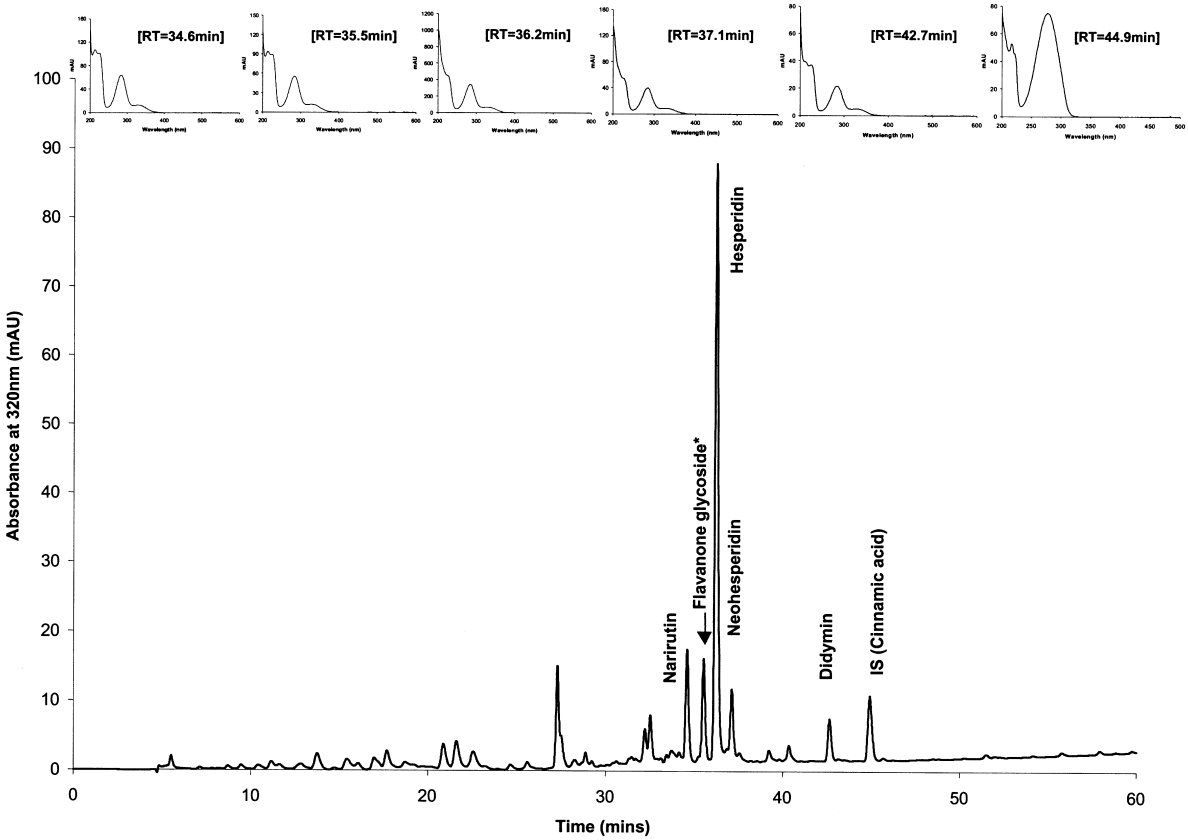

FIGURE 2 Representative HPLC-DAD (DAD monitored over the range 200–750 nm) chromatogram of a flavanone-rich extract, i.e. orange (conditions of analysis as in text). Peaks were derived at 320 nm and identified as follows: narirutin [$RT = 34.6$ min], flavanone glycoside [$RT = 35.5$ min], hesperidin [$RT = 36.2$ min], neohesperidin [$RT = 37.1$ min], didymin [$RT = 42.7$ min] and IS (cinnamic acid) [$RT = 44.9$ min].

are also the monomeric flavan-3-ols, i.e. (+)-catechin and (−)-epicatechin. Although the analytical approach reported here was able to separate and identify catechin and epicatechin by selecting the wavelength at 280 nm, the quantification of the corresponding chromatographic peaks was difficult and not reliable due to the small size of the peak in complex chromatograms since most of the components in the fruit and vegetables extracts have high absorbance values at 280 nm. This problem was also encountered for other fruit extracts which contained flavan-3-ols (such as apples, peaches, raspberry, strawberry, plums, pears) and therefore no data relative to concentrations of flavan-3-ols are reported here.

Hydroxycinnamate-rich Fruits and Vegetables

The components of hydroxycinnamate-rich fruit and vegetables, e.g. apple, pear, peach and tomato, are listed in Table VI, with a typical HPLC trace for apple extracts in Fig. 4. Chlorogenic acid (5′-caffeoyl quinic acid) was identified as the major phenolic component [$RT = 17.5$ min] in pear (5.07 mg/100 g FW), apple (3.26 mg/100 g FW) and peach/nectarine (4.46 mg/100 g FW). The other major identified components in apple extracts were rutin [$RT = 33.3$ min] (2.76 mg/100 g FW), other flavonol conjugates [$RT = 35.3$ and 35.7 min] (2.64 mg/100 g FW) and phloridzin [$RT = 36.9$ min] (2.32 mg/100 g FW). Pear extracts also were found to contain quercetin-3-glucoside and other flavonol conjugates; the total quercetin-3-glucoside/conjugates concentration was calculated to be 2.56 mg/100 g FW on the basis of quercetin-3-glucoside. Tomato extracts also contained rutin (1.94 mg/100 g FW), a component that was putatively identified as chalconaringenin [$RT = 45.6$ min] (2.98 mg/100 g FW), but no naringenin or naringenin glucosides were found. Significant amounts of cyanidin-3-glucoside and/or other cyanidin conjugates [$RT = 23.2$ min] (3.47 mg/100 g FW), present in the peel of the nectarines, and 3′-caffeoylquinic acid [$RT = 10.5$] (1.77 mg/100 g FW), were found in peach extracts.

Very low amounts of flavonoids were found in other fruit and vegetables: no major phenolic component was identifiable by our means in peas, cauliflower and red cabbage (data not shown) and only after enzyme hydrolysis was it possible to measure minimal amounts of ferulic acid in banana extracts, and ferulic and sinapic acids, in cauliflower extracts (data not shown).

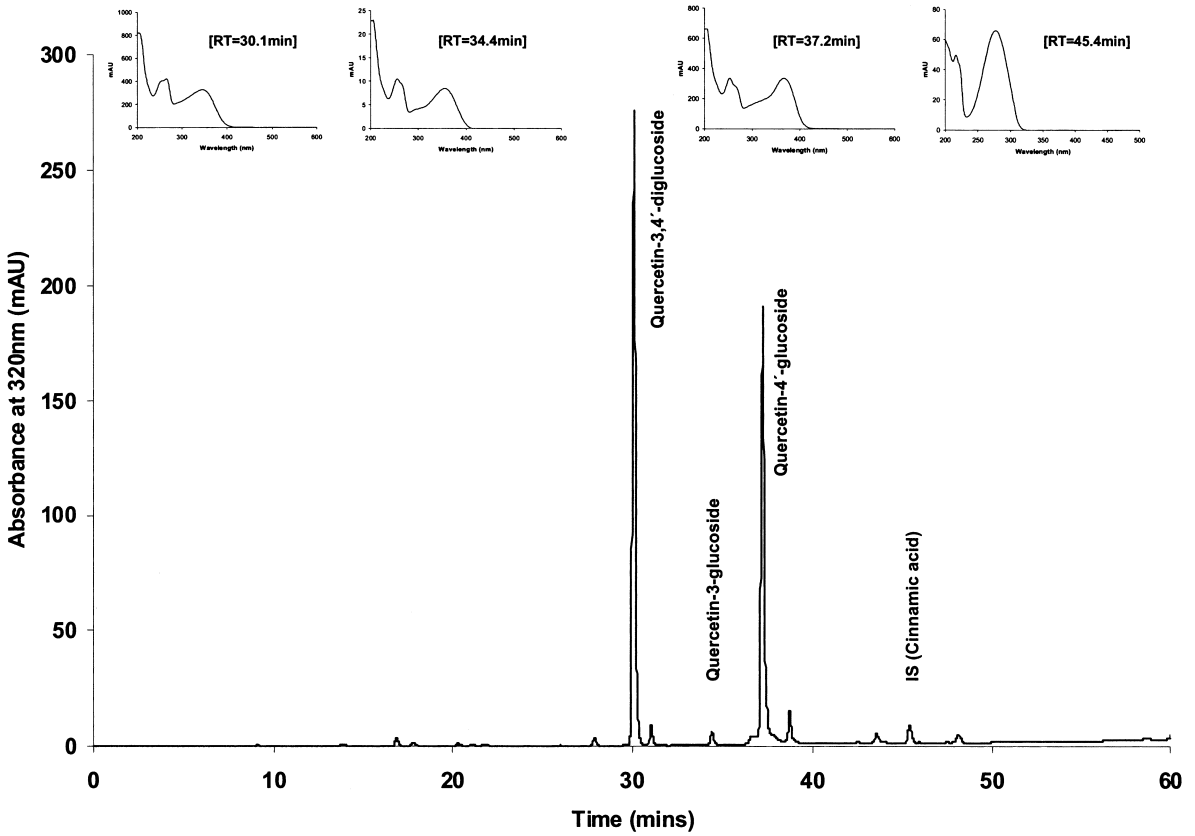

FIGURE 3 Representative HPLC-DAD (DAD monitored over the range 200–750 nm) chromatogram of a flavonol-rich extract, i.e. onion (conditions of analysis as in text). Peaks were derived at 320 nm and identified as follows: quercetin-3,4′-diglucoside [$RT = 30.1$ min], quercetin-3-glucoside [$RT = 34.4$ min], quercetin-4′-glucoside [$RT = 37.2$ min] and IS (cinnamic acid) [$RT = 45.4$ min].

Total Phenolics and Vitamin C

The total phenolic and vitamin C contents and the TEAC, FRAP and ORAC values of fruit and vegetables extracts are reported in Table VII. In Fig. 5 the observed association between total phenolics (A) and vitamin C (B) content and measurements of antioxidant activity *in vitro*, TEAC, ORAC and FRAP, are presented.

The amount of total phenolic in the fresh berry fruits analysed here ranged between 228 and 330 mg/100 FW, as gallic acid equivalents (GAE). The highest total phenolic content was found for strawberry extracts (330 mg/100 g FW). Similar amounts were observed for red plum extracts (320 mg/100 g FW) while raspberry extracts had the lowest content (228 mg/100 g FW). Strawberry extracts showed the highest ascorbic acid content (54 mg/100 g FW) and antioxidant activity (TEAC: strawberry ⩾ red plum = raspberry) among the berry fruit and all the other fruit and vegetable extracts analysed here (Table VII).

The phenolic and vitamin C contents were similar for orange and grapefruit extracts (total phenolics: 126 and 150 mg/100 g FW, respectively; vitamin C: 46 and 52 mg/100 g FW, respectively), although grapefruit reported the highest levels. A similar trend was observed in the TEAC values while orange extracts had higher antioxidant activity than grapefruit extracts according to the FRAP and ORAC values (Table VII).

In the flavonol-containing fruit and vegetables, the amount of total phenolics ranged from 14 to 88 mg GAE/100 g FW. The highest phenolic content (128 mg GAE/100 g FW) was observed for broccoli extracts which also showed the highest Vitamin C content (9 mg/100 g FW). The antioxidant activity of broccoli extracts was also high but lower than that elicited by spinach extracts. Lettuce extracts had the lowest phenolic content (14 mg/100 g FW), contained a very low amount of vitamin C (<2 mg/100 g FW) and showed little antioxidant activity (Table VII). The hierarchy of antioxidant activities, as indicated by the TEAC values, in the flavonol-containing fruit and vegetables group was the following: spinach > broccoli > green grape ≅onion > green cabbage⩾leek > lettuce. The total phenolic contents of hydroxycinnamate-rich fruit and vegetables were considerably lower than those measured for the anthocyanin-, flavanone- and flavonol-rich products (range: 30–60 mg GAE/100 g FW). Also the vitamin C contents were lower (<2 mg/100 g FW in pear, apple, banana and cauliflower, 3 mg/100 g FW in peach and 14 mg/100 g FW

TABLE V Major phenolic components identified by HPLC-DAD (mean±SEM of a minimum of three samples) in green grape, onion, leek, lettuce, broccoli, spinach and green cabbage extracts

Component (mg/100 g FW)	Green grape	Onion	Leek	Lettuce	Broccoli	Spinach	Green cabbage
Quercetin conjugates*	–	65.14±1.55*†	–	16.79*‡	–	–	
Quercetin-3-glucoside	1.14±0.03	0.76±0.02	–	–	–	–	–
Kaempferol conjugates*	0.19±0.01*¶	–		–	–		14.98±2.22*‡
Flavonol conjugates*	–	–	91.23±18.06*‡	–	7.12±1.28*‡§	52.52±8.93*‡	–
5′-Caffeoylquinic acid	–	–	–	6.68	–	–	–
Other Hydroxycinnamate Conjugates*	0.24±0.01*‖	–			16.44±1.92*‖,#	21.09±2.88*‖	44.15±6.23*‖
Anthocyanin conjugates*	–	–	–	2.49***	–	–	–

* Identification implied from PDA spectra and literature data.
† Quercetin-3,4′-diglucoside and quercetin-4′-glucoside;[45].
‡ Quantified on the basis of quercetin-3-glucoside.
¶ Quantified on the basis of kaempferol-3-glucoside.
§ Quercetin-3-sophoroside and kaempferol-3-sophoroside;[50].
‖ Quantified on the basis of *p*-coumaric acid.
1,2′-disinapoyl-2-feruloyl gentiobiose, 1-sinapoyl-2-feruloyl gentiobiose, 1,2,2′-trisinapoyl gentiobiose and 1,2-disinapoyl gentiobiose;[46].
** Quantified on the basis of cyanidin-3-glucoside.

in tomato) and the antioxidant activities were in the order apple > pear > tomato≅peach=leek > banana (Table VII).

DISCUSSION

For this study, several varieties of each fruit and vegetable, produced in different countries and available from UK supermarkets, were mixed in order to minimise the influence of the cultivar, growing soil and sunlight exposure on the compositional and quantitative data.[39] In addition, it is likely that the country of origin and the variety of the fresh fruit and vegetables in the normal diet vary widely according to the season and the availability.

Although a number of techniques and methods for the separation of one or more different classes of phenolic compounds have been developed,[40] the method applied here has the advantage of a simple and rapid extraction procedure and the use of the same HPLC system for the optimal and simultaneous separation and detection of four major classes of phenolics: anthocyanins, flavanones, flavonols and hydroxycinnamates. The levels of anthocyanins, flavanones, flavonols and hydroxycinnamic derivatives reported were generally within the ranges expected from a variety of literature data for specific fruit and vegetables, although a direct comparison was often difficult due to the range of methods, variety of fruit and vegetables used and the unit in which the results were expressed.

In addition, the compounds are detected and reported in their glycosidic forms where possible, as they occur in the diet. This is for two reasons: acid hydrolysis to aglycones can induce degradation unless conditions relevant to the specific flavonoid family are devised and applied. Secondly, the β-glucosidase enzyme treatment can allow further elucidation of specific glucosylated phenolics, although enzyme treatment leads to loss of reproducibility since protein binding and precipitation of the less water-soluble phenolics can occur (therefore aglycones post-enzyme treatment could not be accurately quantified; data not reported).

Anthocyanin-rich Fruit

The results indicate that fruits such as strawberry and raspberry are an excellent source of phenolics and vitamin C and therefore possess an extremely high antioxidant potential. In fact, the anthocyanins, which are the major class of phenolics in this type of fruits, generally have demonstrated high antioxidant activity in *in vitro* systems.[19,36,41,42] The data reported here are essentially in the same range as reported findings. Levels of anthocyanins in strawberry, red raspberry and plum have been reported in

TABLE VI Major phenolic components identified by HPLC-DAD (mean±SEM of a minimum of three samples) in pear, apple, tomato, peach and banana extracts

Component (mg/100 g FW)	Pear	Apple	Tomato	Peach	Banana
5′-Caffeoylquinic acid	5.07±0.20	3.26±0.07	2.19±0.04	4.46±0.08	–
3′-Caffeoylquinic acid*		–	–	1.77±0.02*	–
Quercetin-3-glucoside/conjugates*	2.56±1.91*†	2.64±0.16*†	0.68±0.01*†	0.77±0.03*†	0.45±0.01*†
Rutin	–	2.76±0.24	1.94±0.05	–	–
Phloridzin	–	2.32±0.08	–	–	–
Naringenin	–	–	0.13±0.01	–	–
Chalconaringenin*	–	–	2.98±0.15*	–	–
Cyanidin-3-glucoside/conjugates*	–	0.83±0.09*‡	–	3.47±0.18*¶	–
Other hydroxycinnamate conjugates*	–	1.28±0.05*‡	1.28±0.05*‡	–	–

* Identification implied from PDA spectra and literature data.
† Quantified on the basis of quercetin-3-glucoside.
‡ Quantified on the basis of *p*-coumaric acid.
¶ Present in the peel.

the range 15–35, 10–60 and 2–25 mg/100 g FW, respectively,[5] and 28–70, 23–59 and 1.9–5.3 mg/100 g FW[1] compared to 11.9, 28.9 and 19.4 mg/100 g FW measured by us. Hertog *et al.*[2] found a mean content of 0.86 and 1.2 mg/100 g FW of quercetin and kaempferol, respectively, after extraction and acid hydrolysis, in strawberry and 0.9 and <0.2 mg/100 g FW of quercetin and kaempferol in plum, compared with our observations of 2.36 and 0.46 mg/100 g FW of quercetin and kaempferol conjugates, respectively, in strawberry, and 2.65 and 1.32 mg/10 g FW in plum. Macheix *et al.*[1] indicated 2.1–17.4, 7.2–10.2 and 2–5.2 mg/100 g FW of flavonols in strawberry, red raspberry and plum, respectively, consistent with the levels reported here.

Flavanone-rich Fruit

Flavanones are characteristic compounds of *citrus* fruit and occur as rutinosides (6-*O*-α-L-rhamnosyl-D-glucosides) and neohesperidosides (2-*O*-α-L-> rhamnosyl-D-glucosides). Rutinosides are characteristic of sweet orange and lemon while neohesperidosides are dominant in bitter orange and grapefruit. The presence of neohesperidin in our compositional data for orange is due to the fact that one of the orange varieties in the mixture was a bitter type (marmalade oranges). The flavanone levels in orange and grapefruit reported here are essentially similar to those reported by others, although mainly levels for citrus juices are actually present in literature. For example, Bronner and Beecher,[43] have reported 120–150 and 24–30 mg/100 g FW of hesperidin and narirutin, respectively, in orange juice concentrates, compared with our mixed varieties of fresh orange fruit which contained 98.5 and 16.1 mg/100 g FW of hesperidin and narirutin, respectively. The same authors also reported a content of 200 and 62–68 mg/100 g of naringin and narirutin, respectively, in grapefruit juice concentrates, in relation to the findings reported here of 197.1 and 29.2 mg/100 g FW naringin and narirutin, respectively, in mixed fresh grapefruit fruit varieties. Justesen *et al.*[44] found an average content of 56.0 mg/100 g FW for hesperetin, the aglycone of hesperidin, and 11.3 mg/100 g FW for naringenin, the aglycone of narirutin, in orange pulp. One early report indicated that the concentration of hesperidin in sweet orange ranges from 270 to 600 mg/100 g FW in the whole fruit and that of naringin in grapefruit from 170 to 280 mg/100 g FW.[1] Published data for various types of citrus juices have recently been summarised, showing extreme content variability in relation to the cultivar and the method of preparation of the juice.[4] Thus, the reported content of hesperidin and narirutin in fresh, hand-squeezed juice from various cultivars of oranges was 122–254 and 18–65 mg/l, respectively. Similarly, levels of naringin and narirutin in grapefruit juice obtained from various cultivars were 73–419 and 23–124 mg/l, respectively.

Flavonol-rich Fruit and Vegetables

Although flavonols, particularly quercetin and its glycosides, are among the most studied flavonoids in terms of biological activities, quantitative data for fruit flavonols are not abundant, because analytical difficulties are often encountered due to the small quantities present in food. In fact, levels of quercetin, the most widespread flavonol aglycone, as determined after acid hydrolysis, are generally below 1 mg/100 g FW except for onions (34 mg/100 g FW), broccoli (3–3.7 mg/100 g FW) and lettuce (1.4–7.9 mg/100 g FW).[6] The data reported here for the concentration of quercetin glycosides in onion extracts are higher than those indicated by Hollman and Arts[6] but essentially in the same range of those reported by Moon *et al.*[45] (40.5, 9.7 and 0.3 mg/100 g FW of quercetin-3,4′-diglucoside, quercetin-4′-glucoside and quercetin-3-glucoside, respectively). The observed differences could in part be related to the use of different varieties and a different analytical

TABLE VII Total Vitamin C (mean of two samples), total phenolics (GAE=Gallic Acid Equivalents), TEAC, FRAP and ORAC (mean±SEM of a minimum of three samples) in fruit and vegetables extracts

Fruit/vegetable	Total phenolics (mg GAE/100 g FW)	Total Vitamin C (mg/100 g FW)	TEAC (μmol Trolox/100 g FW)	FRAP (μmol Fe^{2+}/100 g FW)	ORAC (μmol Trolox/100 g FW)
Strawberry	330±4	61	2591±68	3352±38	2437±95
Raspberry	228±6	26	1846±10	2325±53	1849±232
Red plum	320±12	5	1825±28	2057±25	2564±185
Grapefruit	150±4	52	861±53	829±6	1447±67
Orange	126±6	46	849±25	1181±6	1904±259
Red cabbage	158±4	37	1377±49	1870±18	2124±68
Broccoli	128±4	45	648±25	833±16	1335±62
Onion	88±1	6	532±29	369±13	988±30
Green grape	80±4	2	594±72	519±48	872±48
Spinach	72±1	7	757±54	1009±35	1655±115
Green cabbage	58±1	28	492±18	694±14	1180±68
Pea	32±1	22	440±l8	251±9	704±62
Cauliflower	30±1	15	295±16	259±5	425±44
Leek	22±1	16	240±11	160±1	413±15
Lettuce	14±1	<2	171±12	124±7	319±37
Pear	60±3	3	282±19	315±24	587±50
Apple	48±1	6	343±13	394±8	560±18
Peach	38±1	6	244±9	336±4	764±49
Banana	38±4	10	181±39	164±32	331±59
Tomato	30±1	18	255±14	344±7	420±39

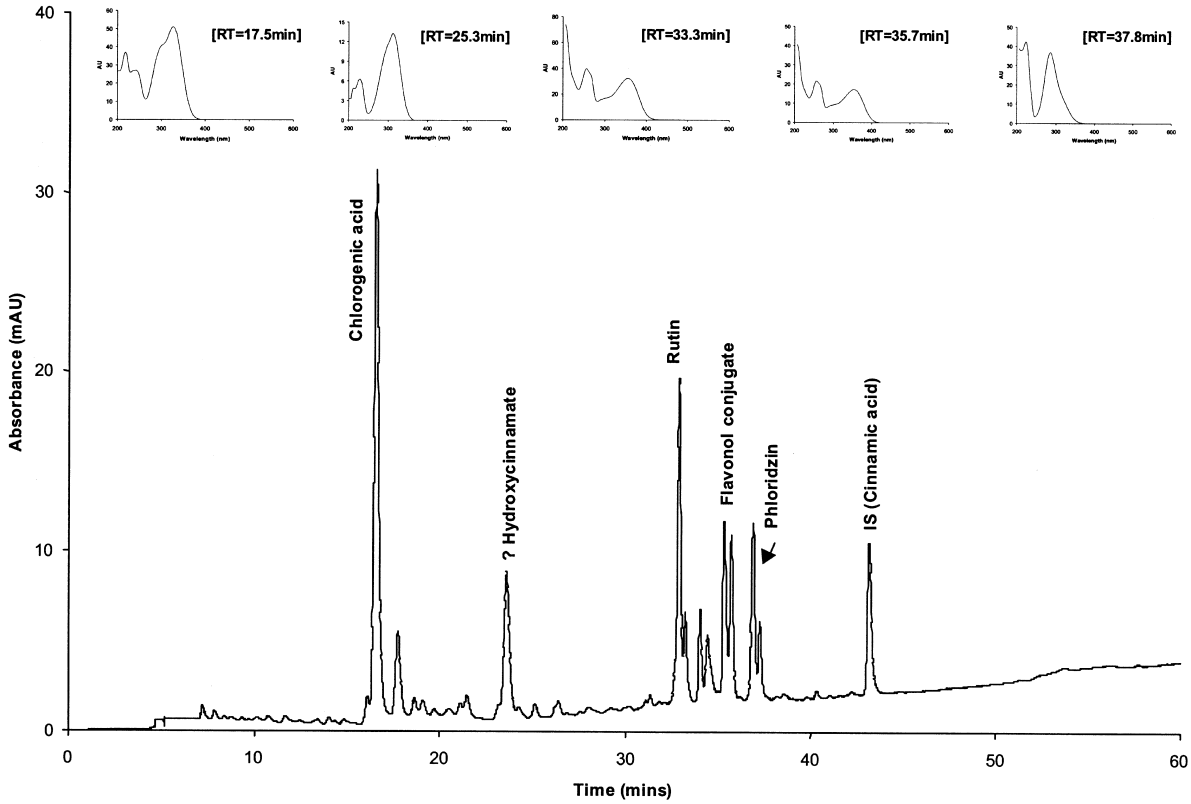

FIGURE 4 Representative HPLC-DAD (DAD monitored over the range 200–750 nm) chromatogram of an hydroxycinnamate-rich extract, i.e. apple (conditions of analysis as in text). Peaks were derived at 320 nm and identified as follows: chlorogenic acid [RT = 17.5 min], hydroxycinnamate [RT = 25.3 min], rutin [RT = 33.3 min], flavonol conjugate [RT = 35.7 min], phloridzin [RT = 37.8 min] and IS (cinnamic acid) [RT = 43.2 min].

approach, as quercetin glycosides in onion extracts have been quantified on the basis of quercetin-3-glucoside and this may have resulted in an over- or underestimation of the relative amounts. In most fruit the quercetin content averaged 1.5 mg/100 g FW, except for apples for which 2–3.6 mg/100 g FW has been reported.[6] In addition, Price *et al.*[46] have found that the total flavonol content, expressed as quercetin aglycone, ranged between 2.64 and 7.39 mg/100 g FW in eight varieties of apples. The data reported here for apples and other flavonol-containing fruit and vegetables were essentially in agreement with previous findings by Hollman and Arts[6] although these authors reported concentrations of the aglycones after acid hydrolysis instead of the naturally occurring glycosidic forms. However, many of the components of green leafy vegetables, such as broccoli, spinach, cabbage and lettuce, could not be precisely identified and quantified by DAD, due to complexity of flavonol glycoside mixtures, in which the glycosides are often acylated with hydroxycinnamic acids.[47–49] Therefore the flavonol levels we have reported for lettuce, broccoli, spinach and green cabbage, which are based on the quantification of the major peaks with flavonol-like spectra such as quercetin-3-glucoside, are likely to be overestimated. In fact, Hertog *et al.*[2] have indicated <1 and <2 mg/100 g FW of quercetin and kaempferol aglycones, respectively, in spinach and green cabbage, compared to our estimation of 52.2 and 15 mg/100 g FW of flavonol conjugates, quantified on the basis of quercetin-3-glucoside, in spinach and green cabbage, respectively. Others have reported the presence in cabbage of 3-*O*-β-D-sophoroside-7-*O*-β-D-glucosides of kaempferol and quercetin, with and without further acylation with ferulic, sinapic and caffeic acids, by using HPLC with mass-spectrometric detection[49] but no indication of levels was given. Similarly, acylated flavonol glycosides and gentiobiosides, derivatives of spinacetin and patuletin, have been isolated from spinach leaves.[47] Thus, on the other hand the levels reported by Hertog *et al.*[2] for spinach and green cabbage might be underestimated: given the elaborate chemical nature of the flavonol glycoside mixtures in spinach and green cabbage, the hydrolysis conditions used might have not been sufficient to break down the complex glycosides to the simple aglycones. Broccoli has been reported to contain about 6 mg/100 g FW of chlorogenic acids and about 2 mg/100 g FW sugar esters.[3] In addition, broccoli florets have been indicated to contain a number of mixed feruloylsinapoyl esters of gentiobiose in high amounts, >30 mg/100 g FW.[50]

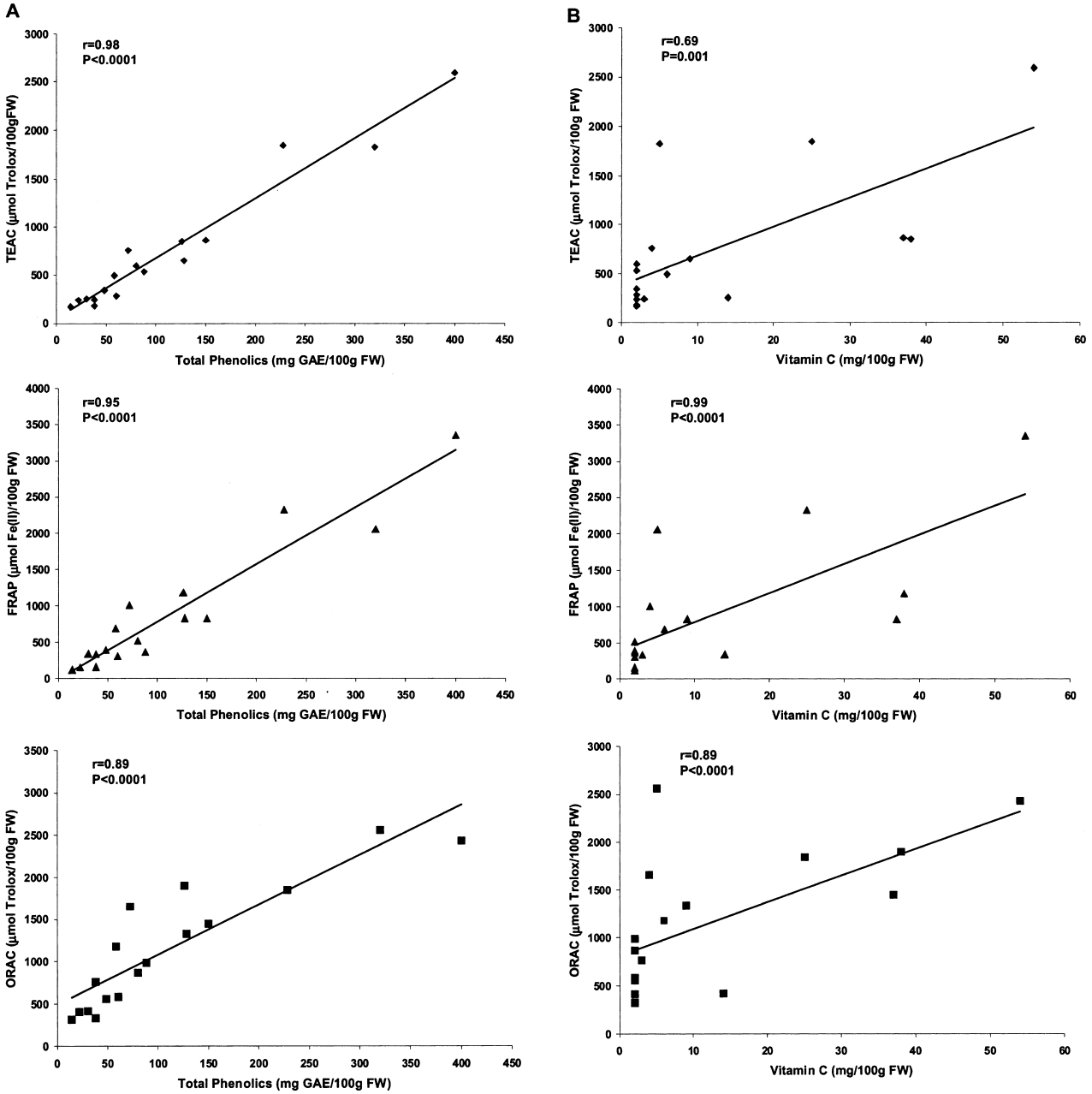

FIGURE 5 Correlation between (A) total phenolics [mg GAE (Gallic Acid equivalents)/100 g FW], (B) Vitamin C (mg/100 g FW) and measurements of antioxidant activity, TEAC, FRAP and ORAC, of fruit and vegetables extracts. The correlation coefficients (r) and the correspondent significance values (P) are indicated.

These findings are in agreement with our estimation of 16.4 and 7.1 mg/100 g FW hydroxycinnamate and flavonol conjugates, respectively, in broccoli. Our data are also in accordance with Hertog *et al.*[2] who have reported a mean content of 3 and 7.2 mg/100 g FW of the aglycones quercetin and kaempferol, respectively. Cauliflower contained no more than 2 mg/100 g FW of combined chlorogenic acids and sugar esters according to Clifford[3] but the methodology reported here was unable to characterise clearly any phenolic component in cauliflower extracts.

Hydroxycinnamate-rich Fruit and Vegetables

Literature data indicate a wide variability in phenolic content of apples. For example, Macheix *et al.*[1] have indicated a total hydroxycinnamate content in the range 6.2–134 mg/100 g FW. Others suggest a mean concentration of 3–6 mg/100 g FW of the specific hydroxycinnamate chlorogenic acids both in the isolated flesh and skin,[3] reflected in a content of 6.2–38.5 mg/100 g FW caffeoylquinic acids in whole apples, with 5-caffeoylquinic acid always dominant. Similarly, a range of 6.4–

51.8 mg/100 g FW hydroxycinnamic acid derivatives in pear have been reported[1] while in the review by Clifford[3] the caffeoylquinic acids, in the whole fruit, ranged from 6 to 28 mg/100 g FW. Thus, the level of chlorogenic acid, 5′-caffeoylquinic acid, measured by us in apples and pears is at the lower end of the reported range. However, in the studies described here we were able to detect 5′-caffeoyl-quinic acid, for which a standard is commercially available, while the levels reported by others appear to include other isomers, such as 4′- and 3′-caffeoylquinic acids, *p*-coumaroylquinic acids, feruloylquinic acids and hydroxycinnamic acids glucose derivatives[1] which we have presented as "Other Hydroxycinnamate Conjugates" in relevant tables. Furthermore, Macheix *et al.*[1] have reported 17.8–40.5 mg/100 g FW flavonols in apple peel. However the level of quercetin glycosides measured by us in whole apple extract was much lower and in agreement with Hertog *et al.*[2] and Justesen *et al.*[44], who have published a 2–3.6 and 2 mg/100 g FW, respectively, quercetin content in apple. Quercetin in pear has been found to be about 0.3 mg/100 g FW by Hertog *et al.*[2] and 4.5 mg/100 g FW by Justesen *et al.*,[44] consistent with our findings of 2.56 mg/100 g FW combined quercetin-3-glucoside and other conjugates. Furthermore, Macheix *et al.*[1] published a level of 4 and 20–60 mg/100 g FW in the fruit and in the peel, respectively. We have also found apple extracts to contain phloridzin (2.32 mg/100 g FW), a dihydrochalcone, in amounts similar to chlorogenic acid and quercetin glycosides. However, it has been asserted that phloridzin in apple is mainly located in seeds and is absent from the fruit but there have also been reports of the presence of phloridzin in apple juice and some data appear to confirm the presence of phloridzin in apple skin in the range 8.7–33 mg/100 g.[1] The levels of quercetin glycosides reported here for tomato extracts (2.62 mg/100 g FW combined quercetin-3-glucosides, rutin and other quercetin conjugates) mirror the range reported by Hertog *et al.*[2] 0.8 mg/100 g FW, by Stewart *et al.*,[51] 0.1–2.2 mg/100 g FW total flavonols (combined free and conjugated quercetin and kaemferol from tomato fruits of different varieties and produced in various country), and by Justesen *et al.*,[44] 1.4 mg/100 g FW.

We also measured relatively high amounts of chalconaringenin but did not detect any naringenin in tomato extracts. This is in agreement with Tomas-Barberan and Clifford[4] whose review reports that naringenin chalcone is present in tomato skin in amounts of about 6.4 mg/100 g FW, and acid hydrolysis, used prior to HPLC analysis, converts the chalcone to the corresponding flavanone, naringenin, which is naturally present only in trace amounts, 0.2–1.5 mg/100 g FW, in tomato.

Total Phenolics, Vitamin C and Antioxidant Activities

The amounts of total phenolic, as determined by the Folin assay, for the fruit and vegetables extracts analysed here, were essentially in the same range of those reported in the literature. For example, Heinonen *et al.*[52] have reported 161–265 mg GAE/100 g FW of total phenolics, depending on the method of extraction, in strawberry extracts and 265–303 mg GAE/100 g FW for red raspberry extracts. As a measure of antioxidant activity, the same extracts were tested by two copper-catalysed *in vitro* assays, inhibition of oxidation of human low-density lipoprotein (LDL) and liposome oxidation. It was found that red raspberry extracts were more effective than strawberry extracts in inhibiting hexanal formation in both the LDL oxidation and the liposome oxidation systems and in inhibiting the formation of hydroperoxides in the liposome oxidation system. In the work reported here, a higher antioxidant activity for strawberry than raspberry extracts was observed. Although this is consistent with the fact that total phenolic content was found to be higher in strawberries than in raspberries, it is also in accordance with our observation that vitamin C is present in significantly higher amounts in strawberry than in raspberry extracts. Kalt *et al.*[53] have determined the total phenolic, anthocyanin and ascorbate content and antioxidant capacity of fresh strawberries and raspberries. They found that strawberry and raspberry extracts contained 86 and 121 mg GAE/100 g FW phenolics and 34 and 21 mg/100 g FW ascorbate, respectively and ORAC values of 2060 and 2140 μmol Trolox equivalents/100 g FW, respectively. The large differences in total phenolic content are probably not surprising when considering that different varieties have been used. Furthermore, Wang and Lin[54] reported that the total phenolic content in different varieties of ripe strawberry fruits ranged from 95 to 152 mg GAE/100 g FW but they also found a variation from 91 to 278 mg GAE/100 g FW depending on the stages of maturity, with the less ripe fruits showing the highest phenolic content. Others[24] have measured only the total antioxidant activity (ORAC) of fruits, with no indication of the phenolic or vitamin C content, and found a hierarchy (strawberry > plum > orange > grapefruit > grape > banana > apple > tomato > pear) closely similar to that reported here (Table VII), although their ORAC values were in a much lower range (134–1536 μmol Trolox equivalents/100 g FW). Consistent with the publication of Wang *et al.*,[24] we found that the antioxidant activities of orange and grapefruit extracts were much lower than those observed for berry extracts.

This is not at all surprising since flavanones have consistently shown lower antioxidant efficiency in terms of H-donating capacity, with respect to anthocyanins and flavonols, when evaluated in *in vitro* systems.[19,21,36] However, the antioxidant potential of orange and grapefruit extracts was appreciably higher than that observed for most of the flavonol- and hydroxycinnamate-rich fruit and vegetables extracts and this is likely to include the higher vitamin C content of citrus fruits.

The vitamin C content of extracts from the flavonol-containing fruit and vegetables was very low and therefore their antioxidant activities were accounted almost exclusively by the phenolic components which was also generally lower when compared to the anthocyanin- and flavanone-rich fruits. Although flavonols, in particular quercetin, possess all the structural features that have been identified as determinants for maximum radical scavenging ability, such as the *o*-dihydroxy structure in the B ring, the 2,3 double bond in conjugation with a 4-oxo function in the C ring and, the 3,5-OH groups with 4-oxo function in the A and C rings, this will be severely modulated by conjugation, e.g. glucosylation on positions 3 and 4′.[21] Vinson *et al.*[55] have analysed various flavonol-containing fruit and vegetable extracts for phenolic content obtaining values similar to those reported here. However these authors used the Folin assay with catechin as standard. The total phenolic content reported by Vinson *et al.*,[55] limited to those vegetables that we have analysed here and expressed as mg catechin equivalents/100 g FW, was 23 for lettuce, 38 for tomato, 49 for spinach, 52 for cabbage, 70 for yellow onion, 104 for broccoli and 116 for red onion. They also tested the antioxidant quality of the extracts by the extent of inhibition of low-density lipoprotein copper-mediated oxidation, obtaining an antioxidant activity hierarchy (onion > broccoli > tomato > spinach > cabbage > lettuce) which is different from that reported here (Table VII). However, this is not surprising since the antioxidant activity of the vegetable extracts in Vinson *et al.*[55] was tested in a lipophilic system, compared with our hydrophilic systems. In fact, Cao *et al.*,[23] who have measured the antioxidant activity of some common vegetables using the ORAC assay, obtained an hierarchy of antioxidant capacity (spinach > broccobroccoli > cabbage > onion > lettuce) very similar to that reported here (Table VII).

Overall, extracts of hydroxycinnamate-rich fruit and vegetables (apples, pears, peaches, tomatoes) analysed in this study showed the lowest phenolic and vitamin C content, reflected in the lowest antioxidant activity, as predicted by the low antioxidant potential of the hydroxycinnamic acids.[21] However, the total phenolic content of French apple varieties has been reported to be in the range 110–600 mg epicatechin equivalents/100 g FW in the cortex of fresh fruits,[56] much higher with respect to the levels observed by us (Table VII). This huge variation may be explained in part by varietal differences and by the use of a different phenol standard. Our findings for total phenolic content in peach extracts are essentially in the range of levels reported by Chang *et al.*[57] of 43–77 mg GAE/100 g FW for eight peach varieties. In the same paper, it was also indicated that the total phenolic content of the peach extracts correlated with their antioxidant activity, as determined by the inhibition of low-density lipoprotein oxidation.

Also in this work, total phenolic and vitamin C contents of the fruit and vegetables extracts analysed showed a good correlation with all measures of antioxidant activity, particularly TEAC, in agreement with previous findings.[51,58,59] Nevertheless, antioxidant activity might not always correlate with amounts of phenolics;[60] but it is important to note that most studies undertaken in this regard have applied pure aglycones and not the dietary forms. Furthermore, as previously mentioned, antioxidants have different scavenging potential depending on whether the radicals are generated in the aqueous or the lipophilic phase.[21,61] In addition, it has to be considered that the Folin–Ciocalteu' assay is subjected to interference from vitamin C;[35] therefore, for extracts particularly rich in vitamin C, for example strawberry, the interference might be extremely significant.

In the work reported here, the overall hierarchy of antioxidant activities in terms of 100 g FW uncooked portion size, as exemplified by the TEAC values, ranged from 2591 to 171 μmol Trolox equivalents, and was as follows: strawberry⩾raspberry=red plum⩾red cabbage⋙grapefruit=orange > spinach > broccoli > green grape≅ onion > green cabbage > pea > apple > cauliflower≅ pear > tomato≅peach=leek > banana≅lettuce. Thus, it can be concluded that those fruit and/or vegetables, which are rich in anthocyanins, flavanones and flavonols, represent the major sources, in terms of activity, of flavonoid antioxidants, as well as vitamin C, in the human diet. By identifying the major bioactive components in foods and by characterising their biological activities, useful information can be obtained to help in defining the role of these dietary components in protection towards a number of diseases, particularly cardiovascular diseases, certain forms of cancer and neurodegenerative diseases. In the work presented here, it has been highlighted that the *in vitro* antioxidant potential of fruit and vegetables plainly reflects their phenolic composition and vitamin C content. However, it should be noted that phenolic compounds may also exert protective effects through other mechanisms, involving influences on signal transduction processes and

gene expression for example.[62–67] Furthermore, the overall functions of flavonoids *in vivo* have yet to be clarified in terms of conjugated or metabolised forms of the dietary phenolics that might be responsible for their biological activities *in vivo*.

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