JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 6301−6306 **6301**

Distribution of Conjugated and Free Phenols in Fruits: Antioxidant Activity and Cultivar Variations

U. IMEH AND S. KHOKHAR*

Procter Department of Food Science, University of Leeds, LS2 9JT, U.K.

Total and free phenolic contents of 16 commonly consumed fruits (comprising 9 apples, 4 pears, and one each of peach, plum, and kiwi fruit cultivars) were measured by Folin-Ciocalteu assay. Total phenol contents varied from 272 to 475 mg of CtE/100 g of fresh weight. Of the apple cultivars studied, Braeburn and Empire had the highest and lowest total phenol content, respectively. The apple cultivars ranked in the following decreasing order: Braeburn > Red Delicious > Crisp Pink > Granny Smith > Royal Gala > Bramley > Golden Delicious > Fuji > Empire. Among pear cultivars, the order was Forelle > Taylor's > Peckham's > Conference. Peach and plum equally had high contents. The percentage of conjugated phenolics ranged between 3 (Red Delicious) and 77% (Empire) of the total phenols. Comparison of different cultivars of the same fruit and between different fruits showed broad variations in both phenolic content and in vitro antioxidant activity; a weak correlation (R^2 = 0.58) was observed between the phenolic content of the fruits and the total antioxidant activity, as estimated by the FRAP assay. The calculated dietary intake of total, free, and conjugated phenols from average per capita consumption of apples and pears in different regions of the U.K. varied between 104−126, 53−64, and 51−62 mg of CtE/day, respectively.

KEYWORDS: Phenols; fruits; apple; pear; plum; kiwi; phenol intake; antioxidant activity; FRAP; cultivars

INTRODUCTION

Phenolic compounds are widely distributed in the higher plants, being found mostly in fruits, vegetables, seeds, herbs, and medicinal plants (*1*). Phenolic contents vary among different cultivars of fruits and vegetables, and within different tissues. The skins of apples and mangoes reportedly contain about two to four times the polyphenolic content of their pulp (*2, 3*). In grapes, on the other hand, about 70% of the total phenol content is concentrated in the seeds, with about 30% in the skins (*4*).

Most phenolic compounds in food are naturally present in conjugated forms; in higher plants, low molecular weight phenols occur as glycosides or esters with sugars or related compounds. Phenols in the free state are normally found only in dead or dying tissues. It is, hence, of metabolic significance that flavanols are widely distributed in plant vegetative tissues in unconjugated forms, while most other groups of flavanoids occur as glycosides. Vinson and co-workers reported that the conjugated fraction varied widely among commonly consumed fruits, from 8.7% in cranberry to as much as 90% in watermelon (*5*).

Phenolic compounds are closely associated with the sensory and nutritional quality of foods, contributing directly or indirectly to desirable or undesirable aroma and taste. In low concentrations, phenolics may protect food from oxidative deterioration; however, at high concentration, they (or their oxidation products) may participate in discoloration of foods, and interact with proteins, carbohydrates, and minerals (*6, 7*). Phenolic compounds are thus good antioxidants and substrates for oxidative browning (although, if uncontrolled, this latter may be detrimental). The action of phenolics as antioxidants is beneficial in food and in biological systems, where they are preferentially oxidized, thereby sparing nutrients (such as vitamin E), body cells and tissues.

Recently, phenols in foods have gained much attention, owing to their antioxidant activities and their possible beneficial implications in human health, a consequence of their demonstrated biological activity in prevention of cancer and cardiovascular diseases (*8−10*). Reliable composition data on phenolics and assessment of their activity are essential for calculating dietary intakes for epidemiological intervention and for planning clinical studies to elucidate health protective aspects of fruits. Results are reported here on commonly consumed fruits and their cultivars.

METHODS AND MATERIALS

Commonly consumed cultivars of fruits (apple, pear, plum, peach, and kiwi) were purchased during the months of April and May, 2001, from a local supermarket in Leeds, U.K. All were of eating quality and without blemishes or damage. Folin-Ciocalteau, 2,4,6-tripyridyl-s-triazine (TPTZ), gallic acid, and catechins (all three compounds were of 98% purity) were purchased from Sigma-Aldrich, Poole, U.K. All other reagents used were of AnalaR grade.

Preparation of Fruit Extracts. Fruits were cleaned, and edible parts were chopped into small pieces and blended under liquid nitrogen in a

* To whom correspondence should be addressed. Telephone: +44 113 2332975. Fax: +44 113 2332982. E-mail: S.khokhar@food.leeds.ac.uk.

10.1021/jf020342j CCC: $22.00
Published on Web 09/28/2002

Table 1. Phenol Contents[a] of Commonly Consumed Cultivars of Fruits (mg of CtE/100 g)

fruit	cultivar		free phenols (dry weight)	free phenols (fresh weight)	total phenols (dry weight)	total phenols (fresh weight)
apple	Braeburn	*Malus pumila*	2171 ± 101	364.7 ± 16.4	2826 ± 105	474.7 ± 13.1
apple	Bramley	*Malus pumila*	2601 ± 111	317.2± 11.9	3018 ± 126	368.2 ± 15.4
apple	Cripps Pink	*Malus pumila*	885 ± 96	170.8 ± 13.5	2127 ± 104	410.5 ± 16.3
apple	Empire	*Malus pumila*	881 ± 78	117.1 ± 11.3	2412 ± 119	320.8 ± 15.8
apple	Fuji	*Malus pumila*	934 ± 89	145.6 ± 16.1	2114 ± 124	330.1 ± 19.4
apple	Golden Delicious	*Malus pumila*	710 ± 41	120.6 ± 7.0	2019 ± 95	343.2 ± 16.3
apple	Granny Smith	*Malus pumila*	1267 ± 91	192.5 ± 16	2455 ± 96	373.2 ± 12.2
apple	Red Delicious	*Malus pumila*	2866 ± 102	430.0 ± 13.0	2963 ± 83	444.4 ± 12.5
apple	Royal Gala	*Malus pumila*	1047 ± 98	156.1 ± 10.6	2515 ± 103	374.7 ± 15.3
pear	Conference	*Pyrus communis*	488 ± 60.5	63.3 ± 8.4	2089 ± 94	271.6 ± 10.5
pear	Forelle	*Pyrus communis*	1194 ± 83	190.1 ± 17.3	2566 ± 63	408.2 ± 10.5
pear	Peckham's	*Pyrus communis*	569 ± 46	96.7 ± 9.6	1795 ± 78	305.2 ± 16.5
pear	Taylor's	*Pyrus communis*	525 ± 36	91.2± 6.8	2209 ± 105	384.3± 18.4
peach	Spring Bell	*Prunus persica*	2365 ± 52	300.4 ± 7.7	2692 ± 107	341.9 ± 21.7
plum	Royal Garnet	*Prunus domestica*	2643 ± 112	413.3± 18.7	3022 ± 87	471.4 ± 13.3
kiwi		*Actinidia chinensis*	698 ± 67	108.1 ± 10.8	1770 ± 61	274.4 ± 9.5

[a] Each value is the mean ± SD of 12 replicates from duplicate extractions.

high-speed blender for 1 min. A weighed portion was lyophilized overnight, and the dry weight was determined. The freeze-dried product was ground to a fine powder using a mortar and pestle, and it was stored at 4 °C for analysis.

Extraction of Free and Total Phenols. A weighed portion (0.5 g) of lyophilate was mixed with 25 mL of 50% methanol/water and heated at 90 °C in a plastic screw-capped tube with intermittent shaking for 2 h to determine the unconjugated ("free") phenols present. Another weighed sample was heated with 25 mL of 1.2 M HCl in 50% aqueous methanol for 2 h at 90 °C to measure the total phenols, and it was stored at 4 °C until it was analyzed (*5*). A minimum of two extractions were carried out.

Measurement of Phenols by Folin-Ciocalteu Assay. Total and free phenols of the fruits were determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex; its intensity at 760 nm increases linearly with the concentration of phenols in the reaction medium (*11*). In this study, gallic acid and catechin were both used as spectrophotometric standards. The phenolic contents of the fruits were determined from calibration equations and were expressed as catechin equivalents (CtE/100 g) and gallic acid equivalents (GAE/100 g).

Measurement of Antioxidant Activity. The FRAP (ferric reducing/antioxidant power) assay was performed according to Benzie and Strain (*12*), as modified by Pulido et al. (*13*). Three solutions were required for the active reagent: first, the buffer of pH 3.6, consisting of 3.1 g of sodium acetate and 16 mL of acetic acid made up to 1 L with water; second, a 10 mmol/L solution of TPTZ in 40 mmol/L hydrochloride acid; and finally, a 20 mmol/L aqueous solution of ferric chloride hexahydrate. The reagent (FRAP solution), prepared freshly each day, involved mixing 25 mL of the first solution with 2.5 mL each of the second and third solutions and heating to 37 °C before use. Measurements involved treating 900 μL of FRAP reagent with 30 μL of added sample or standard (or water for blank) and 90 μL of H_2O. Aqueous solutions of known ferrous ion concentration in the range 100−1000 μL (ferrous sulfate heptahydrate) were employed for calibration. Absorbance (*A*) readings were recorded every 10 s at the wavelength 593 nm for 10 min (standards) and then for as long as the reaction is continued in sample experiments. A minimum of three runs were performed on each standard.

RESULTS AND DISCUSSION

Phenol Contents. Expressed on a fresh weight basis, Braeburn apple was found to possess the highest total phenol content (475 mg/100 g); plum (471 mg/100 g) and Red Delicious apple (444 mg/100 g) were also rich sources (**Table 1**). The lowest phenol contents were found in Conference pear (272 mg/100 g) and kiwi fruit (274 mg/100 g) (**Table 1**). Among the nine apple cultivars, phenol contents decreased in the order: Braeburn > Red Delicious > Cripps Pink > Royal Gala ≥ Granny Smith > Bramley > Golden Delicious > Fuji > Empire. For the pears analyzed, phenol content decreased in the order: Forelle > Taylor's > Peckham's > Conference. The single cultivar of plum had a higher total phenol content than most of the apples, and all the pears, studied. On the basis of dry matter, the phenol content of Royal Garnet plum was the highest among the 16 fruits analyzed. Donovan et al. have also concluded that plum has a higher polyphenol content than most fruits (*14*).

Red Delicious apple contained almost all phenolics in the free form while about 80% of the phenolics in Empire were conjugated (**Figure 1**). The distribution of conjugated phenols further varied widely (12−76%) among the other fruits reported here. Vinson and colleagues (*15*) reported a similar variation in the pattern of free and conjugated phenol distributions in fruits. The degree of conjugation is of considerable biological significance. It has been reported that the degree of glycosylation significantly affects the antioxidant properties of the compounds. For example, aglycones of quercetin and myricetin were observed to be more active than their corresponding glycosides in bulk methyl linoleate (*15*), while quercetin from onions, mostly present in the conjugated form, has been reported to be well absorbed in humans (*16*).

Comparison of phenol values obtained in this study with those of other studies would be important; however, differences in spectrophotometric standards employed and in units reported makes direct comparison difficult. To overcome such barriers, two different standards were employed in this study and results were expressed on both a dry and fresh weight basis. The comparisons using different spectrophotometric standards are presented in **Table 2** to demonstrate the variation of measured total phenol content (fresh weight basis) according to the standards employed. An overestimation of about 10% was observed when gallic acid was used as compared to catechin, in agreement with other research (*3*).

The present findings are consistent with those of other researchers; for example, Escarpa and Gonzalez (*3*) reported the total phenol content of Golden Delicious apples was 320 and 370 mg of catechin and gallic acid equivalents, respectively (expressed per 100 g of fresh weight), while Eberhardt et al. (*2*) found the total phenol content of the Red Delicious variety to be 290 and 220 mg of phenolics/100 g of apples with and without skin, respectively. In this study, the total phenol content

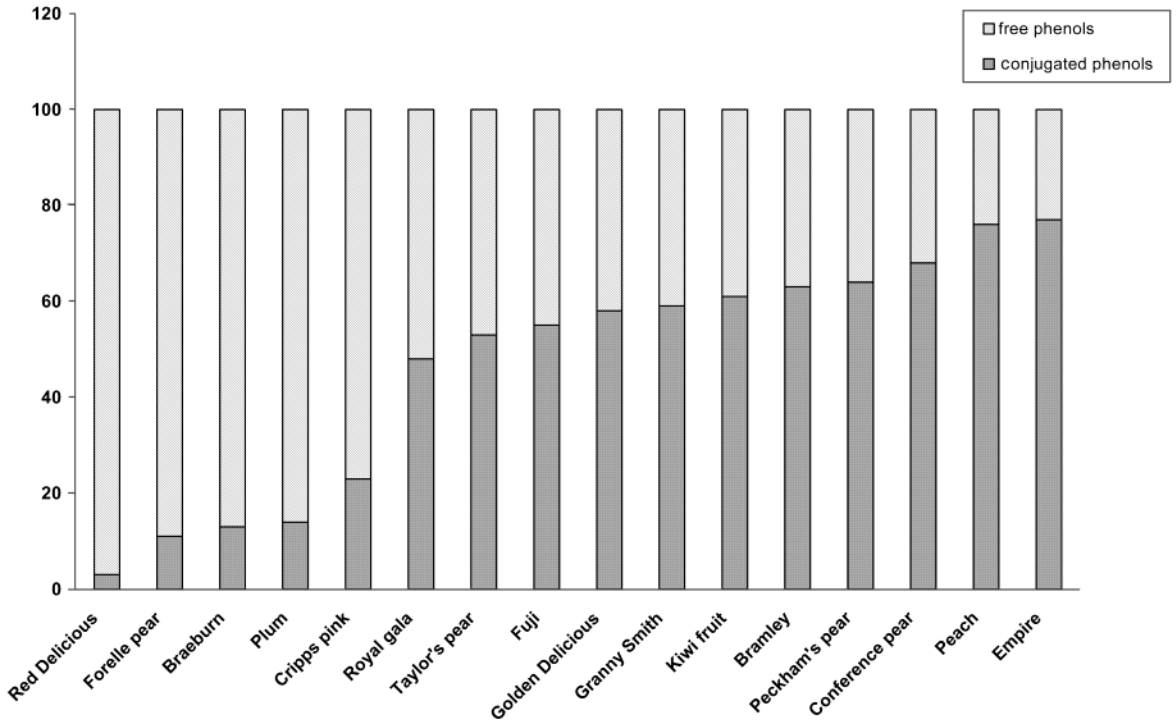

Figure 1. Percent distribution of free and conjugated phenols in fruits.

Table 2. Comparison of Total Phenols[a] by Different Spectophotometric Standards

fruit	cultivar	% dry matter	total phenols (mg of CtE/100 g)	total phenols (mg of GAE/100 g)
apple	Braeburn	15	474.7 ± 13.1	535.0 ± 14.6
apple	Bramley	13	368.2 ± 15.4	415.9 ± 18.0
apple	Cripps Pink	17	410.5 ± 16.3	457.4 ± 14.0
apple	Empire	16	320.8 ± 15.8	359.9 ± 11.8
apple	Fuji	17	330.0 ± 14.4	367.3 ± 12.6
apple	Golden Delicious	16	343.2 ± 13.3	381.5 ± 10.9
apple	Granny Smith	19	373.2 ± 22.2	418.4 ± 13.0
apple	Red Delicious	12	444.4 ± 12.5	500.7 ± 14.7
apple	Royal Gala	16	374.7 ± 15.3	420.5 ± 17.9
pear	Conference	14	271.6 ± 10.5	302.3 ± 15.5
pear	Forelle	17	408.0 ± 10.5	458.2 ± 16.0
pear	Peckham's	16	305.2 ± 12.5	337.1 ± 11.6
pear	Taylor's	15	384.3± 18.4	430.0 ± 21.5
peach	Spring Bell	17	341.9 ± 11.7	384.8 ± 15.2
plum	Royal Garnet	16	471.4 ± 13.3	534.8 ± 16.0
kiwi		13	274.4 ± 9.5	302.8 ± 11.0

[a] Each value is the mean ± SD of 12 determinations. Total phenols are expressed as milligrams per 100 g of fresh weight of fruits.

of the latter was found to be higher (444 mg of CtE/100 g of fresh weight). Kahkonen and co-workers[1] have reported 1200 mg of GAE/100 g of dry weight of unspecified cultivars of apple, rather lower than that observed for any apple (2019−3018 mg of CtE/100 g) in the present study. A likely reason may be that the apples were cored prior to extraction, thus excluding the peel phenolics, which may contribute significantly towards the total apple phenolic content.

In a study of 25 apple cultivars (*17*), Empire was found to be lowest in phenol content, a similar result to that observed in this study. Phenolics have been shown to decrease on a dry weight basis during the seasonal development of fruits and leaves with respect to their ontogenesis, but the single compounds did not behave uniformly (*18*). A shift in flavanol pools from monomeric to oligomeric structures during fruit growth dictated the biosynthetic tendency towards the formation of procyanidins (4β,6-epicatechin) at the end of the growing period. Other researchers suggest that phenolics may vary from season to season (27−300 mg/100 g of fresh weight) in harvested apples because of varied agronomic conditions (*19*).

Estimated U.K. Dietary Intake of Phenolics. The daily intake was calculated on the basis of average per week consumption (*20*) of apples and pears in England (235 g), Wales (225 g), Scotland (195 g), Great Britain (230 g), and Northern Ireland (215 g) and the average phenolic content of apples and pears (see **Table 1**). The average phenolic content of 9 apple and 4 pear cultivars (total phenol 370 ± 56.5, free phenol 189 ±112.7, and conjugated phenol 181 ± 77.6 mg of CtE/100 g of fresh fruit) was used to estimate the dietary intakes. The phenolic intake in different regions in the U.K. varied between 104 and 126 (total phenols), 53 and 64 (free phenols), and 51 and 62 mg (conjugated phenols) mg of CtE/day (**Figure 2**). However, the respective coefficients of variation (CVs) were 15.3, 59.6, and 42.9%, indicating much wider variations in free and conjugated phenols as compare to total phenols mainly because of wide variation in the phenolic content of cultivars and fruits. Free phenolics constituted approximately 50% of the daily intake, suggesting that their higher bioavailability compared to that of conjugated phenolics from the fruits. In comparison, the estimated U.K. dietary intake of total phenols from tea has recently been calculated to be 618 mg of GAE and 555 mg of CtE/day on the basis of an average consumption of 3 cups (600 mL) and calculation of the phenolic content of black teas (*21, 22*). Therefore, the average dietary intake of total phenolics from apple, pear, and tea in the U.K. could be estimated to be as much as 677 mg of CtE/day.

In 1976, Kahnau estimated an average daily intake of flavonoids in the United States to be 1 g/day as quercetin by summing the contribution of individual compounds in foods assessed by thin-layer chromatography and spectrophotometry (*23*). Using HPLC analysis, investigators found the average intake of five flavonoids (quercetin, kaempferol, myricetin,

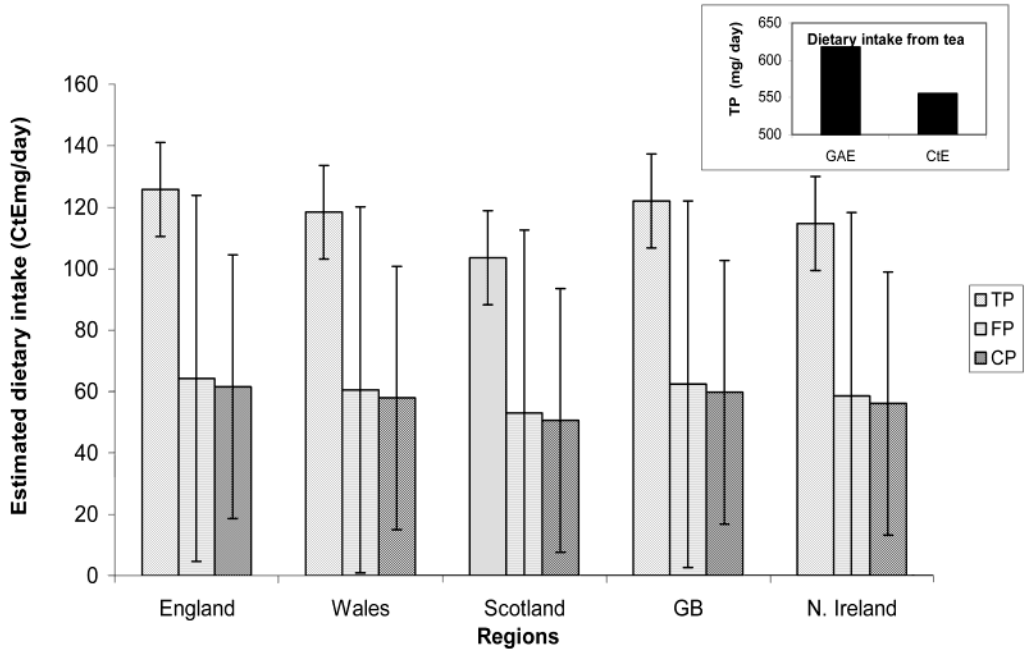

Figure 2. Estimated dietary intake of total (TP), free (FP), and conjugated phenols (CP) in the United Kingdom. Data are given on the basis of average daily per capita consumption of apple and pear together (*20*) and tea (*21*).

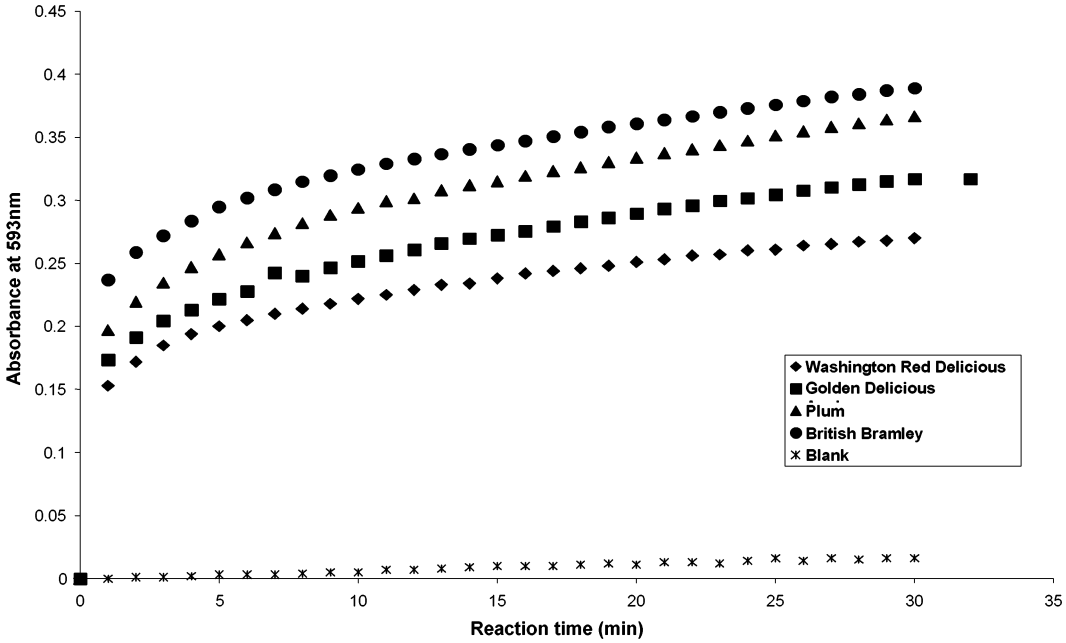

Figure 3. Example of FRAP reaction kinetics of apples and plum extracts. Each value is the mean of four replicates from duplicate extraction.

luteolin, and apigenin) to be 23 mg/day in the Dutch diet (*24*) and 12.9 mg/day in that of the United States (*9*). Recently, an American group reported a flavonoid intake of 20.1 mg/day (*25*) while the Finnish diet has been reported to contain 38.4 mg/day on the basis of 24 flavonoids consumed from fruits and berries, the latter being a rich source of these compounds (*26*). The most recent intake study, reported on the basis of FC phenolics in the U.S diet, has found 255 and 218 mg of CtE/day from fruits and vegetables, respectively (*5*). These reports, considered together, emphasize the difficulty in directly comparing data due to variations in techniques used and the units of expression, and they suggest that it may be appropriate to include total phenolic intake from specified foods in addition to reports on individual or groups of compounds.

Antioxidant Activity. The total antioxidant activities of the fruits are expressed as FRAP values; an example of FRAP kinetics of different fruits is shown in **Figure 3**. Sample FRAP values are interpolated from regression equations (**Table 3**). The total antioxidant activity (TTA) increased significantly in all the extracts between 4 and 30 min; most fruits exhibited an increase in excess of 50%, in agreement with the results of other authors (*13*). The fact that antioxidants in the samples retained their activity, and even showed an increase, might suggest an ability to maintain activity over longer time periods and, thus, may assist maintenance of antioxidant status in vivo. In foods, this might imply an ability to offer protection over a longer period and so prevent early deterioration.

Table 3. FRAP Values (mmol/100 g of fresh weight) at 4, 10, and 30 min

		FRAP value		
fruit	cultivar	4 min	10 min	30 min
apple	Braeburn	1.81 ± 0.23[a]	2.25 ± 0.31	2.89 ± 0.35
apple	Bramley	1.45 ± 0.1	1.76 ± 0.14	2.18 ± 0.19
apple	Cripps Pink	1.27 ± 0.17	1.65 ± 0.23	2.29 ± 0.34
apple	Golden Delicious	1.48 ± 0.27	1.86 ± 0.29	2.42 ± 0.24
apple	Empire	1.10 ± 0.18	1.40 ± 0.21	1.87 ± 0.21
apple	Fuji	1.21 ± 0.27	1.51 ± 0.16	2.01 ± 0.19
apple	Granny Smith	1.10± 0.27	1.39 ± 0.29	1.83 ± 0.29
apple	Red Delicious	1.31 ± 0.27	1.61 ± 0.37	2.01 ± 0.46
apple	Royal Gala	1.06 ± 0.09	1.39 ± 0.12	1.92 ± 0.16
pear	Conference	1.04 ± 0.03	1.28 ±0.08	1.62 ± 0.14
pear	Forelle	1.43 ± 0.22	1.80 ± 0.29	2.25 ± 0.37
pear	Peckham's	1.27 ± 0.12	1.56 ± 0.18	1.94 ± 0.22
pear	Taylor's	1.16 ± 0.13	1.44 ± 0.18	1.83 ± 0.22
peach	Spring Bell	1.07 ± 0.15	1.36 ± 0.14	1.76 ± 0.13
plum	Royal Garnet	1.60 ± 0.15	2.04 ± 0.19	2.61 ± 0.24
kiwi		0.93 ± 0.08	1.15 ± 0.11	1.57 ± 0.10

[a] Each value is the mean ± SD of 4 replicates and is based on fresh weight.

As is evident from **Table 3**, Braeburn exhibited the highest activity at all three times, followed by plum and Golden Delicious; in contrast, kiwi fruit showed the least total antioxidant activity for 30 min. Generally, total activity appeared to increase with time, though the rate of increase gradually decreased, as the reaction kinetics show.

It is interesting that plum exhibited a higher activity than the other fruits, with the exception of Braeburn apple. In an ORAC assay, the antioxidant activity of fruits has been rated in the order: plum > kiwi > apple > pear (cultivars unspecified) (*27*). On average, the present results suggest a higher total antioxidant activity in apples than in pear, with kiwi exhibiting the lowest activity.

Comparison of the TAAs of the various cultivars of apple showed activities in the order: Braeburn > Golden Delicious > Bramley > Red Delicious > Cripps Pink > Fuji > Granny Smith > Empire > Royal Gala; the pear cultivars exhibited a decreasing order: Forelle > Peckham's > Taylor's > Conference. However, the order of total activity differed from that for total phenol content. Correlation analysis showed only a weak correlation ($R^2 = 0.518$) between total polyphenols and total antioxidant activity for all 16 fruits. Literature reports on the relationship between total phenols and antioxidant activities are contradictory; while some authors have observed a high correlation (*1*), others found no direct correlation (*17*). The results of this study indicate that factors other than polyphenols may contribute to total antioxidant activity in these fruits, the most likely being ascorbic acid and beta-carotene. This may suggest that the composition of fruits for other possible vitamin antioxidants (including phenolics) should be considered to investigate if there is any correlation between total antioxidant activity and bioactive compounds.

CONCLUSION

Epidemiological studies should reflect total phenol content and total antioxidant activity of fruits and other plant foods that constitute a diet. Such data will provide a more complete description of the intakes of these antioxidant compounds as compared to a limited number of compounds. The data from the present study clearly emphasize the existence of wide variations among the cultivars.

Therefore, it is essential that compositional studies take into consideration the various factors (agronomic, genomic, pre-and postharvest conditions, and processing) that may affect the chemical composition of plant foods, in general, and that may have a significant role in determining the phenolic composition and the bioactivity of these compounds, in particular. In some population groups, not only is the consumption of fruits and vegetables seasonal but also these may be consumed at different ripening stages; the fruits can also be ripened at home, and the methods employed often vary markedly from standard techniques. Further studies are in progress in our laboratory to examine foods consumed by ethnic populations in the U.K.

The issue of metabolism requires that caution is needed in interpreting the findings of in vitro bioactivity, as examined here, to an in vivo situation. New information is forthcoming on how and when polyphenols are metabolized after absorption and on how much the bioactivities of these metabolites differ from those of the parent molecule. Future studies focusing on functionality of metabolites will be an important step forward in establishing the biological role of the phenolics ingested by humans.

LITERATURE CITED

(1) Kahkonen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J. P.; Pihlaya, K.; Kujala, T. S.; Heinonen, M. Antioxidants of plant extract containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.

(2) Eberhardt, M. V.; Yong, C.; Hei, I. R. Nutrition: Antioxidants activity of fresh apples. *Nature* **2000**, *405*, 903−904.

(3) Escarpa, A.; Gonzalez, M. C. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometer methods. *Anal. Chim. Acta* **2001**, *427*, 111−127.

(4) Waterhouse, A. L.; Walzem, R. L. Nutrition of grape phenolics. In *Flavonoids in health and in disease*; Rice-Evans, C. A., Packer, L., Eds.; Marcel Dekker Inc.: New York, 1998; pp 359−385.

(5) Vinson, J. A.; Yong, H.; Xuehui, S.; Ligia, Z.; Bose, P. Phenol antioxidant quantity and quality in food: fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315−5321.

(6) Shahidi, F.; Naczk, M. *Food phenolics: Sources, Chemistry, effects and application*; Technomic Publishing Group: Lancaster, PA, 1995.

(7) Robarbs, K.; Prenzler, P.; Tucker, G.; Swatsitang, P.; Glover, W. Phenolic compounds and their roles in oxidation processes in fruits. *Food Chem.* **1999**, *66*, 401−436.

(8) Block, G.; Patterson, B; Subar, A. Fruit, vegetables, and cancer prevention; a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1−29.

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

(10) Pearson, D. A.; Tan, C. H.; German, J. B.; Davies, P. A.; Gershwin, M. A. Phenolic contents of apple inhibit human low-density lipoprotein oxidation. *Life Sci.* **1999**, *66*, 1913−1920.

(11) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidative substrates by means of Folin-Ciocalteau reagent, Packer, L. *Methods Enzymol.* **1999**, *299*, 152−178.

(12) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant" Power: The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(13) Pulido, R.; Bravo, L.; Saira-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified FRAP assay. *J. Agric. Food Chem.* **2000**, *48*, 3396−3402.

(14) Donovan, J. L.; Meyer, A. S.; Waterhouse, A. L. Phenolic composition and antioxidant activity of prune and prune juice (Prunus domestica). *J. Agric. Food Chem.* **1998**, *46*, 1247−1252.
(15) Hopia, A. I.; Heinonen, M. Comaprison of antioxidant activity of flavonoids aglycones and their glycosides in methyl linoleate. *J. Am. Oil Chem. Soc*. **1999**, *76*, 139−144.
(16) Hollman, P. C. H.; van der Gaag, M. S.; Mengelers, M. J. B.; van trijp, J. M. P.; de Varies, J. H. M.; Katan, M. B. Absorption and disposition kinetics of dietary antioxidant quercetin in man. *Free Radical Biol. Med.* **1996**, *21*, 703−707.
(17) Eberhardt, M. V.; Liu, R. H.; Smith, N. L.; Lee, C. Y.; de mas Latrie, I. Antioxidant activity of various apple cultivars. *Abstract of papers of The American Chemical Society*; American Chemical Society: Washington, DC, 2001; Vol. 221, part 1, 118-AGFD.
(18) Mayr, U.; Treutter, D.; Santos-Buelga, C.; Bauer, H.; Feucht, W. Developmetal changes in the phenol concentrations of Golden Delicious apple fruit and leaves. *Phytochemistry* **1996**, *38* (5), 1151−1155.
(19) www.naturalhub.com.
(20) Consumption of fresh fruit by Government Office Region, 2000; www.defra.gov.uk/esg/m_survey.htm.
(21) The Tea Council, 2001; www.teacouncil.co.uk.
(22) Khokhar, S.; Magnusdottir, S. G. Total phenol, catechin and caffeine contents of commonly consumed teas in the UK. *J. Agric. Food. Chem.* **2002**, *50* (3), 565−570.
(23) Kuhnau, J. The flavonoids. A class of semi-esssential food components; their role in human nutrition. *World Rev. Nutr. Diet.* **1976**, *24*, 117−191.
(24) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease; the Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007−1011.
(25) Rimm, E. B.; Ascherio, A.; Giovanucci, E.; Speigelman; Stampfer, D.; Willet, W. C. Vegetable, fruit and cereal fibre intake and risk of coronary heart disease among men. *J. Am. Assoc*. **1996**, *275*, 447−451.
(26) Kumpulainen, J. T.; Salonen, J. T. Trolox equivalent antioxidant capacity of average flavonoid intake in Finland. In *Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease*; Salonen, J. T., Ed.; Royal Society of Chemistry: Cambridge, U.K., 1999; pp 141−150.
(27) Wang, H.; Cao, G.; Prior, R. L. Total antioxidant Activity of Fruits. *J. Agric. Food Chem*. **1996**, *44*, 701−705.

Received for review March 20, 2002. Revised manuscript received July 24, 2002. Accepted July 29, 2002.

JF020342J