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Phenol Antioxidant Quantity and Quality in Foods: Fruits

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The free and bound phenols have been measured in 20 fruits commonly consumed in the American diet. Phenols were measured colorimetrically using the Folin−Ciocalteu reagent with catechin as the standard after correction for ascorbic acid contribution. On a fresh weight basis, cranberry had the highest total phenols, and was distantly followed by red grape. Free and total phenol quality in the fruits was analyzed by using the inhibition of lower density lipoprotein oxidation promoted by cupric ion. Ascorbate had only a minor contribution to the antioxidants in fruits with the exception of melon, nectarine, orange, white grape, and strawberry. The fruit extracts' antioxidant quality was better than the vitamin antioxidants and most pure phenols, suggesting synergism among the antioxidants in the mixture. Using our assay, fruits had significantly better quantity and quality of phenol antioxidants than vegetables. Fruits, specifically apples and cranberries, have phenol antioxidants that can enrich lower density lipoproteins and protect them from oxidation. The average per capita consumption of fruit phenols in the U.S. is estimated to be 255 mg/day of catechin equivalents.

Keywords: *Phenols; antioxidants; fruits; lipoprotein oxidation*

INTRODUCTION

Consumption of fruits and vegetables is associated with a lowered risk of cancer and cancer mortality (*1*). Block and colleagues have shown an inverse association with fruits and vegetables in 128 of 156 separate cancer epidemiology studies, with fruits having a stronger association (*2*). The National Cancer Institute and the National Research Council recommend at least five servings of fruits and vegetables daily. Only 17% of 15000 Americans surveyed at schools, work sites, churches, or nutrition clinics eat this much (*3*). Abundant evidence exists for fruits and vegetables decreasing the risk of heart disease in the U.S. (*4*). Strokes in men participating in the Framingham study were decreased by eating fruits and vegetables (*5*). Citrus fruit and juice were especially protective for decreasing the risk of an ischemic stroke in the Health Professionals Follow-up Study (*6*). An additional benefit of fruits and vegetables is their negative association with blood pressure (*7*). A prospective study of diet quality and mortality in women showed a significant inverse correlation of mortality with increasing consumption of healthy food (*8*).

One possible reason for this protection against diseases (including cancer, cardiovascular, and cerebrovascular diseases) is the presence of antioxidant vitamins C and E, and the provitamin beta carotene, in these foods. One mechanism by which fruits may be beneficial is by providing these vitamin antioxidants. However, recent supplementation studies with pure vitamin E and beta carotene have cast doubt on this hypothesis for men with heart disease (*9*). A 4-year vitamin E supplementation to more than 9000 patients at high risk for cardiovascular events produced no significant benefit (*10*). Daily supplementation for six years with vitamin E or beta-carotene to cigarette smokers showed an increased risk of hemorrhage but a decreased risk of cerebral infarction (*11*). An 8-year prospective study of over 44000 healthy men in the Health Professionals Follow-up Study found no benefits of vitamin C or E for reducing the risk of stroke (*12*).

It has been hypothesized that oxidation of low and very low density lipoproteins (LDL and VLDL, respectively) are crucial steps in atherosclerotic lesion formation (*13*). Oxidation of another target molecule DNA is an important event in carcinogenesis. Indeed, the consumption of a diet high in fruits has been shown to decrease oxidative damage of DNA bases in humans (*14*). Other dietary components, polyphenolic antioxidants such as flavonoids, are reported as protective for heart disease (*15*) and for stroke in the Zutphen Study conducted in The Netherlands (*16*). A Finnish study of 10000 men and women over a 20-year period showed a decreased risk of lung cancer as a result of flavonoid intake (*17*). The intake of apples, the major dietary source of flavonols in this population, was inversely associated with lung cancer incidence. This benefit was not due to the intake of the antioxidant vitamins. A diet rich in fruits and vegetables has recently been found to favorably affect serum antioxidant capacity and protect against lipid peroxidation (*18*). Human consumption of 200 g of strawberries has been shown to increase the plasma antioxidant capacity (*19*). This could not be explained solely by the vitamin C from the strawberries.

We have recently shown that many phenols and polyphenols are stronger antioxidants than the vitamin antioxidants, using as a model the oxidation of the lower density lipoproteins LDL+VLDL (*20*). Phenols have also been found to enrich these lipoproteins (*21*) after spiking in plasma. They thus can provide protection when the lipoproteins penetrate the endothelium of the aorta

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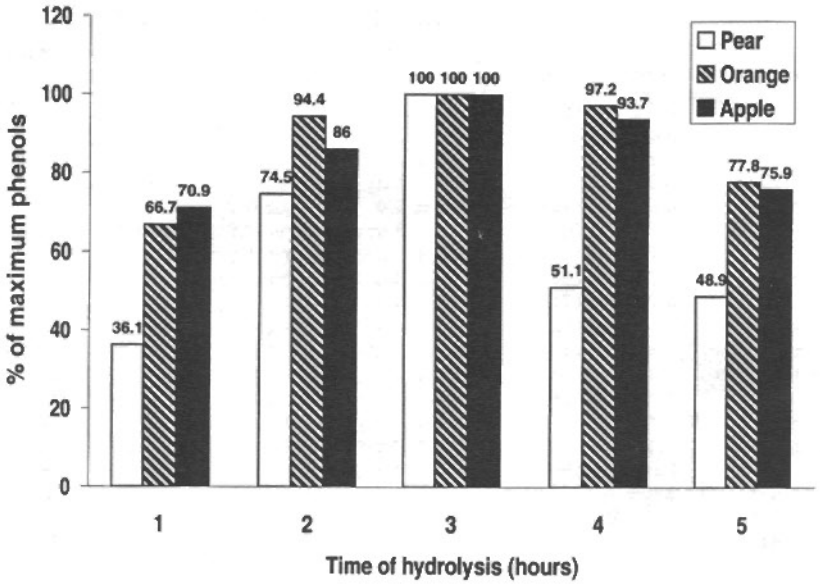

Figure 1. Kinetic study of the acid hydrolysis of 3 representative fruits.

where they are subsequently oxidized (*13*). We have thus investigated the quantity and quality of the phenols present in commonly consumed fruits in the American diet.

MATERIALS AND METHODS

Sample Preparation. Two or three samples of fresh fruits were obtained from local supermarkets. After the fruits were cleaned with tap water and dried, the edible portion was weighed, chopped, and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for one minute. Then a weighed portion (50−100 g) was lyophilized for 48 h (Virtis model 10-324) and the dry weight was determined. The sample was ground to pass through a 0.5-mm sieve and stored at −20 °C until analyzed.

Extraction and Hydrolysis. A 50-mg aliquot of lyophilysate was accurately weighed in a screw-capped tube. We used a procedure similar to those described for vegetables (*22*) and other food extracts (*23*). For free phenols, 5 mL of 50% methanol/water and the sample were vortexed for one minute and heated at 90 °C for 3 h with vortexing every 30 min. After the samples were cooled, they were diluted to 10 mL with methanol and centrifuged for 5 min at 5000 rpm with a benchtop centrifuge to remove solids. Total phenols were extracted with 5 mL of 1.2 M HCl in 50% methanol/water and treated as above.

Analysis. Phenols were measured in duplicate samples of each fruit at 750 nm using the Folin−Cocialteu reagent diluted 5-fold before use (Sigma Chemical Co., St. Louis, MO), with catechin as the standard and measurement at 750 nm after reaction for 10 min. Ascorbic acid was measured in the free phenols extract after diluting with 5% metaphosphoric acid to 10 mL. HPLC was done with a 25-cm 10-μm C_{18} column (Supelco, Inc., Bellefonte, PA) using a solvent of 86% water/4% acetic acid/10% methanol with a flow rate of 2 mL/min at 254 nm. To calculate the per capita consumption of phenols from fruits, the most recent fruit consumption data from 1997 were used in the calculations (*24*).

The quality of the phenol antioxidants was measured by determining the IC_{50} (the concentration to inhibit oxidation 50%) of the pooled phenol extracts for each fruit. Phenol extracts were added to affinity-column isolated LDL+VLDL at concentrations ranging from 0.2 to 2 μM followed by a standard oxidation with cupric ions at physiological pH and temperature. The oxidation mixture was reacted with thiobarbituric acid, and the products were measured by fluorometry in butanol. A native sample without cupric ions and a blank sample without an antioxidant were also analyzed. All samples were done in duplicate. This method has recently been described in detail (*23*). The phenol antioxidant index (PAOXI), a combined measure of quantity and quality of phenol antioxidants (*25*), was determined on the total phenol extracts by dividing the phenol concentration (μmol/kg) by the IC_{50} value (μM).

The ability of phenols from fruits to enrich LDL+VLDL in plasma and protect them from subsequent oxidation was measured in two representative fruits. Plasma was spiked with the methanol/water extract of apples and cranberries (50 and 100 μM), along with a control, and equilibrated for 1 h at 37 °C. The LDL+VLDL was isolated by affinity column and oxidized with cupric ions under standard conditions which include a physiological pH and temperature. The kinetics of conjugated dienes formation were determined at 234 nm, and the lag times (where the initial slow oxidation line converges with the rapid oxidation line) were measured. The method has previously been described in detail (*23*).

RESULTS AND DISCUSSION

In an attempt to optimize the conditions for extraction and hydrolysis, three of the fruits were subjected to a kinetic study (Figure 1). Three hours was chosen for the optimal time. The phenol contents of the 19 fruits are given in Table 1. The polyphenol concentrations of the fruits are measured as both free and total phenols. Only a few fruits (avocado, cranberry, honeydew melon, and orange) have a large portion of their phenols in a free form; the other fruits have a high percentage of the phenols conjugated, ranging from 31 to 94%. This result is similar to our vegetable data. Vegetables showed a range of 23 to 87% in the conjugated form (*22*). Cranberries had by far the largest amount of both free and total phenols among the fruits, with red grapes a distant second. Citrus fruits have a very low concentration. The rankings are not meant to be taken too literally as the results in Table 1 show that for many fruits there is a large variation in phenol content. A popular measure of antioxidant quantity in fruits is the oxygen radical assay (ORAC) which uses a fluorescent assay by means of a centrifugal analyzer (*26*). ORAC measures the oxidation of a water-soluble protein substrate phycoerythrin with a synthetic organoperoxy radical generator, and our method monitors the oxidation of lipoproteins with cupric ion which forms natural lipid alkoxy

Table 1. Total Phenol Content of Fruits on the Basis of Dry and Wet (Fresh) Weight

fruit	total phenols[a] (μmol/g) dry weight	total phenols[a] (μmol/g) wet weight (rank)	free phenols (μmol/g) dry weight	free phenols (μmol/g) wet weight	mole % free phenol Folin antioxidants as ascorbate[b]	% phenols conjugated[b]
apple	34.1 ± 4.8	6.4 ± 0.9 (9)	16.4 ± 2.3	3.1 ± 0.4	1.7%	51.9%
avocado	12.7 ± 4.7	3.5 ± 1.3 (11)	10.3 ± 3.8	2.8 ± 1.1	8.8%	18.9%
banana	42.3 ± 8.1	11.2 ± 2.1 (3)	12.6 ± 2.4	3.4 ± 0.1	4.6%	70.2%
blueberry	62.0 ± 7.8	8.9 ± 1.1 (5)	13.2 ± 1.5	1.9 ± 0.3	16.5%	78.7%
cantaloupe	8.1 ± 4.2	0.9 ± 0.5 (19)	2.6 ± 1.4	0.3 ± 0.2	9.7%	67.9%
cherry	52.3 ± 7.3	10.9 ± 1.5 (4)	24.6 ± 3.4	5.1 ± 0.8	14.0%	53.0%
cranberry (frozen)	158.8 ± 3.2	22.7 ± 0.5 (1)	140.9 ± 13.1	21.0 ± 1.5	2.9%	8.7%
grape (red)	63.7 ± 41.2	13.6 ± 8.8 (2)	33.5 ± 6.6	7.2 ± 2.3	2.0%	47.4%
grape (white)	52.3 ± 31.6	6.7 ± 4.0 (7)	8.0 ± 3.7	1.0 ± 0.7	73.6%	84.7%
grapefruit	7.5 ± 2.9	0.9 ± 0.3 (20)	4.2 ± 1.8	0.5 ± 0.2	13.5%	44.0%
lemon	19.6 ± 0.4	2.4 ± 0.1 (13)	10.2 ± 0.2	1.3 ± 0.0	6.4%	48.0%
melon (honeydew)	11.4 ± 4.7	1.3 ± 0.5 (18)	1.7 ± 0.7	0.2 ± 0.1	84.0%	85.1%
nectarine	12.3 ± 2.1	1.5 ± 0.3 (16)	5.0 ± 0.8	1.0 ± 0.2	30.5%	59.3%
orange	18.9 ± 10.7	1.4 ± 0.8 (17)	3.6 ± 1.6	0.3 ± 0.1	73.5%	81.0%
peach	27.9 ± 7.7	2.4 ± 0.7 (12)	9.7 ± 2.5	0.8 ± 0.2	22.4%	65.2%
pear	41.4 ± 4.9	6.6 ± 0.7 (8)	19.0 ± 2.2	3.0 ± 0.4	6.4%	54.1%
pineapple	11.9 ± 6.0	2.3 ± 1.2 (14)	7.0 ± 3.6	1.4 ± 0.8	5.4%	41.2%
plum	58.2 ± 5.2	7.8 ± 0.7 (6)	39.1 ± 3.6	5.4 ± 0.5	0.7%	31.1%
strawberry	72.3 ± 11.0	4.6 ± 0.7 (10)	39.1 ± 4.5	2.5 ± 0.4	40.3%	45.9%
watermelon	19.5 ± 8.0	2.2 ± 0.9 (15)	1.9 ± 1.1	0.21 ± 0.12	9.5%	90.3%

[a] Total phenols assayed in the hydrolyzed sample. [b] The value is identical for the wet and dry samples.

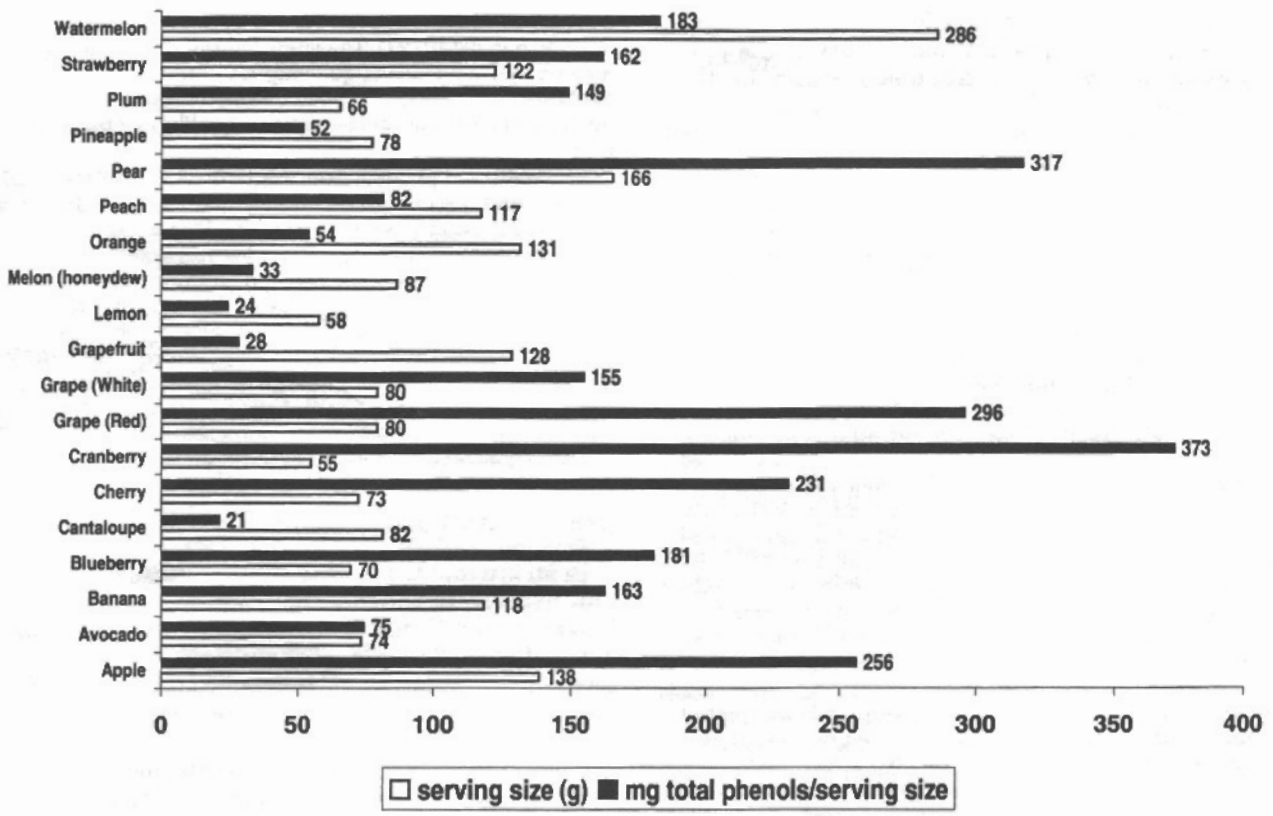

Figure 2. Amount of total phenols in fruits (as catechin equivalents) on the basis of serving size.

and peroxy radicals (*27*). Using the ORAC assay, blueberry has the highest amount of antioxidant activity among the fruits, and cranberry was somewhat less. Our assay found cranberry considerably higher in free phenols than blueberry by fresh weight. Although ORAC and Folin give different values for different fruits and vegetables, they correlate significantly with each other (*22*).

Comparing the fruits on the basis of serving size is also useful because there is a very large difference in serving size. For instance, the smallest serving size is 55 g for cranberries and the largest is 286 g for a wedge of watermelon. Total phenols in fruits on the basis of serving size are shown in Figure 2. The order of the top 10 is cranberry > pear > red grape > apple > blueberry = watermelon > banana = strawberry > white grape > plum. The berries provide the largest amount of phenols according to serving size among the fruits.

Comparing our calculated fresh weight phenols with literature data in which individual phenols have been measured is useful. For instance, sweet cherries were found to contain two phenolic acids and five anthocyanins for a total of ~6 μmol/g (*28*). Our value was 10.9 μmol/g catechin equivalents of free phenols. In blueberries, a total of 9.3 μmol/g was found (*29*) in the form of hydroxycinnamates (as caffeic acid), flavanols (as cat-

Table 2. Antioxidant Quality (IC_{50}) and Total Phenol Antioxidant Index (PAOXI) of Fruits

	IC_{50}(μM)		total[a] PAOXI × 10^{-3}			
			dry		wet	
fruit	free	total[a]	weight	rank	weight	rank
apple	0.21	0.31	110	8	20.6	8
avocado	0.51	0.21	60.5	14	16.7	9
banana	0.70	0.39	108	9	28.7	7
blueberry	0.13	0.22	273	4	40.5	3
cantaloupe	0.21	0.25	32.4	20	3.6	20
cherry	0.08	0.10	523	2	109	1
cranberry	0.86	0.75	212	6	31.2	6
grape (white)	0.75	0.20	262	5	33.5	5
grape (red)	0.73	0.27	351	3	50.3	2
grapefruit	0.49	0.19	39.5	18	4.7	17
lemon	0.22	0.29	67.6	10	8.28	13
melon (honeydew)	0.19	0.31	36.8	19	4.2	18
nectarine	0.78	0.19	64.7	12	7.89	14
orange	0.95	0.34	55.6	15	4.1	19
peach	0.56	0.46	60.7	13	5.22	15
pear	0.34	0.51	81.2	11	12.9	11
pineapple	0.72	0.27	44.1	17	8.52	12
plum	0.70	0.50	116	7	15.6	10
strawberry	0.12	0.12	603	1	38.3	4
watermelon	0.26	0.44	44.3		5.00	16

[a] Total phenols analyzed in the hydrolyzed extract.

echin), flavonols (as rutin), and anthocyanins (as malvin). Our analysis of free phenols was 8.9 μmol/g. This group found 1.8 μmol/g of these same classes of compounds in strawberries and we measured 4.6 μmol/g of free phenols. Lemons were analyzed for individual phenols by HPLC and the total dry weight phenols were 24 μmol/g (*30*), whereas we found 19.6 μmol/g.

Because ascorbate gives a Folin reaction (an oxidation−reduction reaction) its concentration was measured separately by HPLC. The retention time of ascorbate was 2.7 min as a sharp peak well-separated from other peaks in the HPLC. Because ascorbate gives a molar response 92% of catechin in our Folin reaction as determined by the extinction coefficient, 92% of the concentration of ascorbate was subtracted from that of the Folin phenols to determine the actual concentration of phenols present in the free phenol extracts. We found additivity of the phenols and ascorbate in our Folin assay by spiking experiments. Ascorbate was destroyed in the total phenols extract under the acid conditions and heat, therefore the total phenol concentration could be determined directly from the Folin assay. As can be seen in Table 1, ascorbic acid is generally a minor component compared with the free phenols present in the fruits. This result was also found by Prior using the ORAC with several berries (*31*). Only white grape, melon, nectarine, orange, and strawberry have more than 25% of their Folin antioxidants as vitamin C.

The quality of the antioxidants in the fruits was determined by calculating the IC_{50}, with the lower numbers indicating the higher quality of antioxidants in the fruits (Table 2). In the free phenol extract, the quality was a sum of the phenols and any vitamin C present. The IC_{50} of ascorbic acid is only 1.45 μM, and therefore its contribution to the quality is small as the phenols in the fruits have much lower values, i.e., are better antioxidants than ascorbate (range 0.08 to 0.78). In general, berries had the best antioxidants among the fruits, with cherry and strawberry being clearly the best for the free and total polyphenol extracts. Cherries, with an IC_{50} of 0.08 μM, equaled the best pure polyphenol, epigallocatechin gallate, the main antioxidant in green tea (*20*). As it was for quantity, fruit quality was

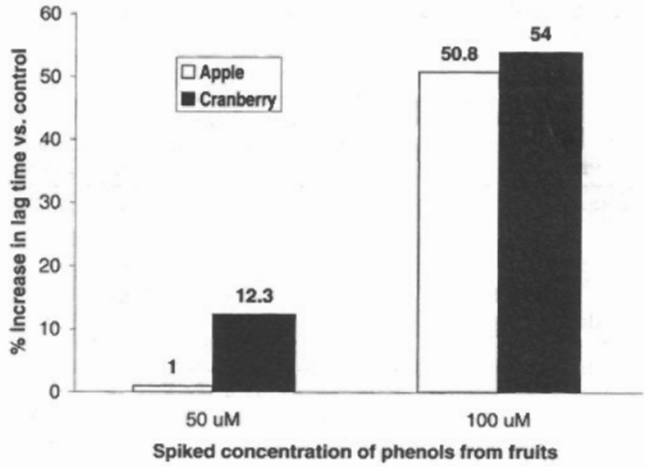

Figure 3. Effect of plasma spiking of fruit extracts on lag time of LDL+VLDL isolated after phenol equilibration and enrichment vs that of a control with no added antioxidants.

also best among the berries. A low value is especially important for the phenols to act as antioxidants in vivo, as their maximal concentrations in plasma are generally less than 1 μM. There was no significant difference between the quality of the free and total phenols in the fruits ($p = 0.08$ by a Wilcoxon signed rank test) although the total polyphenol quality was higher than that of the free phenols: mean 0.32 and 0.48 μM, respectively. In comparison, fruit phenols, both free and total, were significantly better antioxidants than vegetables ($p < 0.005$) (*22*). The fruit extracts' antioxidant quality was also superior to vitamin antioxidants which range from 1.45 μM for ascorbate to 4.30 μM for beta carotene, and also superior to most pure phenols (*20*). This suggests synergism among the antioxidants in the mixture, such as that found with wine (*32*).

The quantity/quality index, PAOXI, is one comprehensive parameter for comparing food antioxidants (*25*). Using this index calculated for total phenols and wet weight (Table 2), cherry is number one distantly followed by red grape, blueberry, strawberry, white grape, cranberry, banana, and apple. Thus berries, among the fruits, are the best source of polyphenol antioxidants. Cherry had the highest wet weight PAOXI of any fruit or vegetable studied. In fact, fruits had signficantly higher PAOXI values than vegetables, averaging 22.4 and 9.2 × 10^3, respectively ($p < 0.05$). One of the reasons for fruits having a better protective effect than vegetables for reducing the risk of chronic diseases such as cancer and heart disease may be the better quantity and quality of the antioxidants in fruits compared to those in vegetables.

Recent studies using compounds spiked in plasma have shown that several types of polyphenols can bind to LDL (*33, 34*). Also, LDL isolated from fasting plasma has been found by GC−MS to contain several flavonols found in the Spanish diet (*35*). The oxidative susceptibility of LDL+VLDL isolated after 26 pure phenols were spiked in plasma and equilibrated was found to be positively correlated with the phenols' protein-binding ability (*36*). The ex vivo lipoprotein-bound antioxidant activity was measured for two representative fruits, apples and cranberries, and the results are shown in Figure 3. Cranberry was a much better antioxidant in this model than apple at the lower concentration (50 μM), but results were similar at the high concentration of 100 μM. The concentration to increase the lag time

Table 3. 1997 Per Capita Consumption of Fruit Phenol Antioxidants in the U.S.

fruit	total phenols[a] fresh weight consumption (mg/100 g)	per capita consumption[b] fresh weight edible portion (g/d)	rank	phenols (mg/d)
banana	335	24.4[d]	(4)	81.8
apple	186	30.7	(2)	57.1
grape[c]	294	9.1	(7)	26.7
watermelon	64	26.7[d]	(3)	17.1
pear	191	8.1	(8)	15.5
cantaloupe	26	35.4	(1)	9.2
peach	70	12.9	(6)	9.0
strawberry	133	6.4	(10)	8.5
orange	41	17.5	(5)	7.2
cherry	316	1.4	(16)	4.4
plum	226	1.9	(14)	4.3
cranberry	678	0.4	(18)	2.7
honeydew melon	38	6.2	(11)	2.4
grapefruit	26	7.6	(9)	2.0
lemon	70	3.4	(12)	2.0
pineapple	67	2.9	(13)	1.9
avocado	101	1.6	(15)	1.6
blueberry	258	0.6	(17)	1.5

[a] Total phenols in the hydrolyzed extract as catechin equivalents. [b] Based on consumption of fresh, canned, dried, and frozen fruit. [c] Average of red and white varieties. [d] Calculation based on 70% of fresh weight is edible (source: USDA).

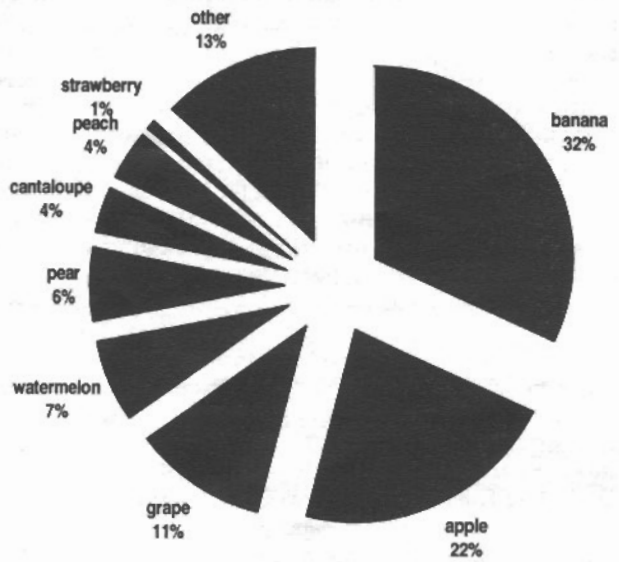

Figure 4. Percent contribution of various fruits to the per capita consumption of fruit phenols.

by 50% (CLT_{50}) was calculated graphically to be 114 μM for apples and 102 μM for cranberries, which is very similar. We have measured CLT_{50} for many pure phenols, vitamins, and beverages (*21*, *37*). A number of substances, such as vitamin E, tea, red wine, grape juice, and chocolate, have been found to enrich the lipoproteins and protect them from oxidation. They were also able to produce an in vivo antioxidant improvement after supplementation in humans (*38*–*43*). Thus, by analogy, we believe that fruit consumption will prove beneficial to protect LDL and VLDL from oxidation in vivo. The antioxidant power of combined phenols was illustrated by a recent study which showed that 100 g of red apples with skins provided antioxidant activity equal to 1500 mg of vitamin C (*44*).

Using HPLC, Dutch investigators found that the average intake of five flavonoids (quercetin, kaempferol, myricetin, luteolin, and apigenin) in the Dutch diet is 23 mg/day (*15*) and in the U.S. diet is 12.9 mg/day (*45*). Using the same method of analysis, an American group recently found flavonoid intake of 20.1 mg/day (*4*). A group from Finland measured 24 flavonoids in foods and calculated that the daily consumption of fruits and berries in the Finnish diet was 38.4 mg/day (*46*). Our results are shown in Table 3. The total for fruits in the U.S. diet is estimated to be 255 mg/day of catechin equivalents. For comparison using our phenol assay, vegetables contribute 218 mg/day (*22*). Bananas provide by far the largest contribution of phenols among the fruits to the U.S. diet. In Finland oranges were first (*46*), and in The Netherlands apples were first (*45*). Looking at Table 3, it is clear that grapes, cherries, plums, blueberries, and cranberries, which have very high concentrations of phenols, are underutilized in the average American diet. The percent contributions of fruits to per capita fruit phenols are shown in Figure 4. Eight fruits (banana, apple, grape, watermelon, pear, cantaloupe, peach, and strawberry) provide 86% of the daily phenols. Epidemiology studies should reflect these new total phenol assay values for fruits and vegetables which give a more complete picture of intake of these antioxidant substances than previous methods which measured a limited number of compounds.

The data show that fruits, and especially berries, provide high quantity and high quality phenol antioxidants that can enrich lower density lipoproteins, thereby protecting them from oxidation. It is thus hypothesized that this antioxidant ability provides some heart disease benefit associated with fruit consumption in epidemiology studies. Animal studies are in progress to determine if fruits, and also vegetables, can inhibit atherosclerosis.

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