Antioxidant Activity of Berry Phenolics on Human Low-Density Lipoprotein and Liposome Oxidation

I. Marina Heinonen,* Anne S. Meyer, and Edwin N. Frankel

Department of Food Science and Technology, University of California, Davis, Davis, California 95616

The antioxidant activity of phenolic compounds present in berries was investigated by two copper-catalyzed in vitro oxidation assays: human low-density lipoproteins (LDL) and lecithin liposomes. The amount of total phenolics varied between 617 and 4350 mg/kg in fresh berries, as gallic acid equivalents (GAE). In LDL at 10 μM GAE, berry extracts inhibited hexanal formation in the order: blackberries > red raspberries > sweet cherries > blueberries > strawberries. In lecithin liposomes, the extracts inhibited hexanal formation in the order: sweet cherries > blueberries > red raspberries > blackberries > strawberries. Red raspberries were more efficient than blueberries in inhibiting hydroperoxide formation in lecithin liposomes. HPLC analyses showed high anthocyanin content in blackberries, hydroxycinnamic acid in blueberries and sweet cherries, flavonol in blueberries, and flavan-3-ol in red raspberries. The antioxidant activity for LDL was associated directly with anthocyanins and indirectly with flavonols, and for liposome it correlated with the hydroxycinnamate content. Berries thus contribute a significant source of phenolic antioxidants that may have potential health effects.

Keywords: *Berries; antioxidants; LDL oxidation; liposomes; flavonoids; hydroxycinnamates; anthocyanins; flavan-3-ols; flavonols*

INTRODUCTION

Epidemiological studies showed that the consumption of fruits and vegetables is related with reduced risk of cancer and cardiovascular disease (Steinmetz and Potter, 1991; Criqui and Ringel, 1994). The flavonoid content of foods is a major dietary factor responsible for this protective effect (Hertog et al., 1993, 1994, 1995; Knekt et al., 1996, 1997). The consumption of onions, apples, tea, and red wine was associated with reduced risk of cardiovascular disease. The consumption of apples was inversely associated with lung cancer incidence and was not due to the consumption of vitamin C or E or β-carotene (Knekt et al., 1997). However, the food compositional data used in these studies were limited mainly to flavonols and flavanones and were restricted in the number of food items. The importance of berries may have thus been underestimated in these epidemiological studies.

Berries are rich in flavonoids and phenolic acids. Most fresh berries contain up to 100 mg/kg flavan-3-ols, up to 300 mg/kg flavonols, up to 200 mg/kg hydroxycinnamates, and relatively high amounts of anthocyanins, especially in the strongly colored blueberries, black currants, crowberries, and sweet cherries (up to 5000 mg/kg) (Herrmann, 1989; Macheix et al., 1990). Higher amounts of flavan-3-ols are found in strawberries, up to 550 mg/kg (Pilando et al., 1985), and blueberries are rich in hydroxycinnamic acid derivatives, up to 2110 mg/kg (Stöhr and Herrmann, 1975). The most abundant hydroxycinnamates are caffeic acid derivatives amounting to 17−41% of the total hydroxycinnamates in black currants, 42−48% in red currants, 90−100% in blueberries, and 35−87% in sweet cherries (Macheix et al., 1990).

Data on the antioxidant activity of berries and fruits, their juices, and berry wines vary widely partly due to the use of different oxidation systems and methods to analyze extracts for phenolic compounds. Extracts of berries of several cultivars of blackberries, black and red currants, blueberries, and black and red raspberries showed a remarkably high scavenging activity toward chemically generated superoxide radicals (Costantino et al., 1992). Black currants were highly active toward xanthine oxidase, possibly due to their high content of anthocyanins and polyphenols. By using an artificial peroxyl radical model system, the extract of fresh strawberries had the highest total antioxidant capacity compared to extracts of plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon (Wang et al., 1996). By using an ascorbate/iron induced lipid peroxidation assay in cell and liver microsomes, a plum extract was the most effective antioxidant followed by peach, grapefruit, apple, and pear (Plumb et al., 1996).

The antioxidant activity of berries and fruits was comparable to that of their juices and wine. Phenolic compounds in fresh grapes were as effective in inhibiting oxidation of human low-density lipoproteins (LDL) as those in red wine (Meyer et al., 1997). Grape juice was shown to exert antioxidant activity in various hydrophilic oxidation models (Kanner et al., 1994; Lanningham-Foster et al., 1995; Abu-Amsha et al., 1996; Wang et al., 1996; Frankel et al., 1998). Anthocyanins were apparently responsible for the antioxidant activity in LDL oxidation both in red wine (Ghiselli et al., 1998) and in grape juice (Frankel et al., 1998). In

* Corresponding author. Visiting scientist, permanent address: Department of Applied Chemistry and Microbiology, P.O. Box 27, Food Chemistry, FIN-00014 University of Helsinki, Finland [fax (+358) 9 708 5475; e-mail Marina.Heinonen@Helsinki.Fi].

10.1021/jf980181c CCC: $15.00
Published on Web 09/12/1998

apple juice most of the antioxidant capacity was reported to be due to chlorogenic acid (32%) or added ascorbic acid (94%) (Miller et al., 1995). Berry and fruit wines made of raw materials such as black currants, crowberries, bilberries, apple, arctic bramble, cowberries, cranberries, red currants, and rowanberries possessed antioxidant activity comparable to that of red wine in inhibiting methyl linoleate hydroperoxide formation (Heinonen et al., 1998a).

The purpose of this work was to study the antioxidant activity of phenolic compounds present in berry extracts using two copper-catalyzed oxidation systems: human low-density lipoprotein in vitro and lecithin liposome. These oxidation models were chosen since LDL oxidation is an early event in coronary disease and liposome oxidation is relevant to oxidation in food systems. Different organic solvents and enzymatic pretreatment prior to extraction were tested for the efficient extraction of phenolic compounds from berries.

MATERIALS AND METHODS

Chemicals. Caffeic acid, chlorogenic acid, catechin, epicatechin, gallic acid, rutin (quercetin 3-rutinoside), hexanal, and Folin and Ciocalteu's phenol reagent were obtained from Sigma Chemical Co., St. Louis, MO. Malvin (malvidin 3,5-diglucoside) was purchased from Pfaltz and Bauer (Waterbury, CT) and L-ascorbic acid from Aldrich Chemical Co. (Milwaukee, WI). Neochlorogenic acid and 3-*p*′-coumaroylquinic acid were gifts from Department of Viticulture and Enology at University of California, Davis. Copper sulfate and HPLC buffers made of ammonium phosphate and orthophosphoric acid were from Fisher Scientific (Fairlawn, NJ), and cupric acetate monohydrate was from EM Science (Cherry Hill, NJ). L-α-Phosphatidylcholine (lecithin from soybean), with phosphatidylcholine content of ca. 40%, was purchased from Sigma Chemical Co. (St. Louis, MO). Pectinex USP was obtained from Novo Nordisk A/S (Bagsværd, Denmark). Grade ACS acetone and methanol and HPLC-grade acetonitrile from Fischer Scientific (Fair Lawn, NJ) were used.

Sample Preparation and Extraction of Phenolics. Blackberries (*Rubus fructicosus*, v. Chester), highbush blueberries (*Vaccinium corymbosum*, v. Jersey), red raspberries (*Rubus idaeus*, v. Tulameen), strawberries (*Fragaria ananassa*, v. Chandler), and two varieties of sweet cherries (*Prunus avium*, v. Bing and v. Burlat) were purchased (2−5 kg) fresh directly from Californian producers. Only the edible part of the fresh berry was used. Berries were homogenized with a Waring blender. The phenolic compounds were extracted from the aliquot (2 g) with 60% aqueous methanol (10 mL) or with 70% aqueous acetone (10 mL) for 2 min using a biohomogenizer rod (Biospec Products Inc., Bartlesville, OK). The efficiency of enzymatic extraction using pectinase was tested with blueberries, strawberries, and sweet cherries. Pectinase was added in concentrations of 1% (also 0.5% to strawberries) of the wet weight of the berry homogenate, and the enzymatic extraction in aqueous, N_2 gassed solution (3−4 mL) was performed at 37 °C for 30 min prior to extraction with the organic solvent. After filtration (Whatman no. 1), the organic solvent was removed by rotary evaporation under vacuum. The extracts were then diluted with double-distilled water to a final volume of 5.0 mL and filtered through a 0.45-μm PTFE filter prior to HPLC and oxidation analysis. All extractions were done in duplicate.

Analyses of Phenolics. The concentration of total phenolics in berry extracts was determined by the Folin−Ciocalteu procedure (Singleton and Rossi, 1965) and is expressed as mg/L gallic acid equivalents (GAE). The phenolic composition of berry extracts was analyzed by HPLC as described by Lamuela-Raventos and Waterhouse (1994). The phenolic compounds were divided into five classes, identified according to their spectral properties, and quantified as follows: flavan-3-ols as catechin equivalents and benzoic acid derivatives as gallic acid equivalents at 280 nm; hydroxycinnamates as caffeic acid equivalents at 316 nm; flavonols as rutin equivalents at 365 nm; anthocyanins as malvin equivalents at 520 nm (Meyer et al., 1997). In addition, ascorbic acid was determined at 280 nm together with the flavan-3-ols and benzoates.

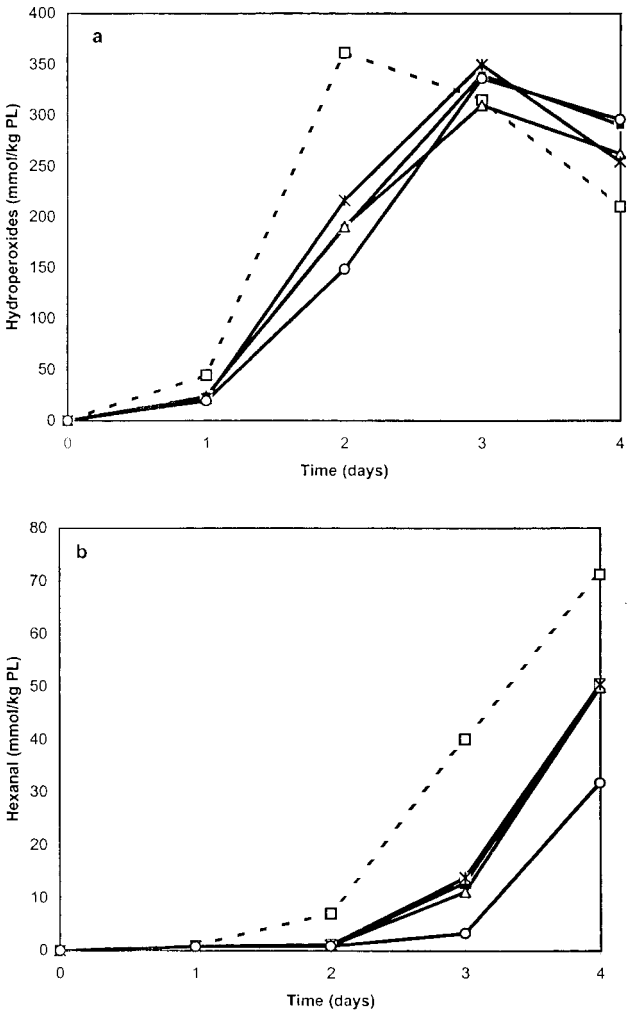

Figure 1. Formation of (a) hydroperoxides and (b) hexanal during lecithin liposome oxidation in the presence of berry extracts at 10 μM GAE: □, control; ■, blackberry; △, blueberry; ∗, strawberry; ○, sweet cherry, Bing.

Isolation of Human LDL. Blood from five normolipidemic people was collected in ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1500*g* at 4 °C to obtain plasma. Plasma LDL was then prepared by sequential ultracentrifugation (Orr et al., 1991) in the presence of 0.01% EDTA and was exhaustively dialyzed and diluted to a standard lipoprotein concentration of 1.0 mg/mL prior to oxidation as described previously (Frankel et al., 1992).

Inhibition of Human LDL Oxidation. The copper-catalyzed (80 μM) oxidation of human LDL was assayed by measuring hexanal by static headspace gas chromatography according to a method developed by Frankel et al. (1992). Aqueous berry extracts extracted with 70% acetone were tested at concentrations of 10 and 20 μM GAE. The results obtained after duplicate analyses were expressed as percent relative inhibition: % In = $[(C - S)/C] \times 100$, where C is the amount of hexanal formed in control and S is the amount of hexanal formed in sample.

Liposome Oxidation Assay. The liposomes were prepared to a final concentration of 0.8 wt % lecithin containing 40% phosphatidylcholine, and the oxidation was followed as described by Huang and Frankel (1997). The average particle size of liposomes was 0.1 μm measured with a Microtrac ultrafine particle analyzer (Leeds & Northrup, North Wales,

Table 1. Inhibition of Formation of Hexanal in in Vitro LDL Oxidation and Inhibition of Formation of both Hydroperoxides (ROOH) and Hexanal in Liposome Oxidation with Phenolic Berry Extracts (70% Acetone) Tested at Concentrations of 10 and 20 μM GAE[a]

	LDL oxidation		liposome oxidation	
berry extract	dilution factor	inh% of hexanal	inh% of ROOH	inh% of hexanal
blackberry, 10 μM	39.3	83.9 ± 0.2 a	41.8 ± 0.1 d	67.8 ± 0.2 e
blueberry, 10 μM	31.5	64.8 ± 1.0 d	38.1 ± 1.7 d	77.1 ± 1.1 c
red raspberry, 10 μM	27.4	78.8 ± 0.2 b	51.8 ± 0.4 c	74.2 ± 2.8 d
strawberry, 10 μM	26.6	53.9 ± 4.1 e	27.4 ± 0.7 e	60.5 ± 0.6 f
sweet cherry, Bing, 10 μM	7.3	70.0 ± 4.5 c	68.8 ± 0.2 a	92.6 ± 0.1 a
sweet cherry, Burlat, 10 μM	7.3	72.7 ± 2.8 c	63.6 ± 1.3 b	82.9 ± 0.3 b
catechin, 10 μM		93.4 ± 0.6	57.4 ± 0.6	41.1 ± 0.2
ascorbic acid, 10 μM		45.2 ± 2.5	−7.4 ± 4.8	2.5 ± 2.9
blackberry, 20 μM	19.7	98.1 ± 0.1 a	66.8 ± 0.7 b	89.3 ± 0.1 b
blueberry, 20 μM	15.8	89.0 ± 0.1 b	41.2 ± 3.1 e	76.7 ± 3.3 d
red raspberry, 20 μM	13.7	98.0 ± 0.1 a	51.5 ± 0.7 d	76.6 ± 2.1 d
strawberry, 20 μM	13.3	86.1 ± 3.8 b	42.8 ± 0.3 e	80.3 ± 0.1 c
sweet cherry, Bing, 20 μM	3.7	99.7 ± 0.2 a	71.6 ± 0.7 a	97.7 ± 0.0 a
sweet cherry, Burlat, 20 μM	3.7	99.9 ± 0.1 a	63.3 ± 0.1 c	88.8 ± 0.3 b

[a] Values in the same column at the same concentration followed by the same letter are not significantly different at $P < 0.05$.

Table 2. Inhibition of Formation of Hexanal in in Vitro LDL Oxidation and Inhibition of Formation of both Hydroperoxides (ROOH) and Hexanal in Liposome Oxidation with Blueberry, Strawberry, and Sweet Cherry Extracts (70% Acetone) Pretreated with Pectinase Tested at Concentrations of 10 and 20 μM GAE

	LDL oxidation	liposome oxidation	
berry	inh% of hexanal	inh% of ROOH	inh% of hexanal
blueberry, 10 μM	60.6 ± 1.9	33.5 ± 0.9	86.1 ± 2.7
strawberry, 10 μM	42.5 ± 3.4	32.0 ± 1.4	24.7 ± 1.5
sweet cherry, Bing, 10 μM	80.7 ± 4.2	73.7 ± 0.3	96.3 ± 0.1
blueberry, 20 μM	83.8 ± 0.3	59.8 ± 1.6	93.2 ± 0.8
strawberry, 20 μM	84.4 ± 2.8	47.7 ± 1.4	64.5 ± 0.5
sweet cherry, Bing, 20 μM	96.7 ± 1.5	75.3 ± 0.4	97.2 ± 0.1

PA). The aqueous berry extracts extracted with 70% acetone were added at concentrations of 20 and 10 μM GAE. The liposome samples were oxidized by addition of cupric acetate (3.0 μM) and shaking at 37 °C in the dark. Duplicate samples for measurements of conjugated diene hydroperoxides and hexanal were taken at 24-h intervals for 5 days. Antioxidant activity was calculated as inhibition: % In = $[(C - S)/C] \times 100$, where C is the amount of conjugated diene hydroperoxides or hexanal formed in control and S is the amount of conjugated diene hydroperoxides or hexanal formed in sample. Inhibition values were calculated at the propagation phase of oxidation, i.e., at day 2 for hydroperoxides and at day 3 for hexanal (Figure 1).

Statistical Analysis. Differences in antioxidant activities and correlations between phenolic composition of berry extracts and their antioxidant activity were tested by one-way analysis of variance and regression analysis, respectively, using Minitab Statistical Software (Addison-Wesley, Reading, MA). Significance level was $P < 0.05$ unless otherwise indicated.

RESULTS

Antioxidant Activity. LDL oxidation was inhibited by 53.9−83.9% by berry phenolics extracted by acetone. At 10 μM GAE the percentage inhibition of LDL oxidation as measured by the formation of hexanal decreased in the following order: blackberries > red raspberries > sweet cherries > blueberries > strawberries (Table 1). At 20 μM GAE, all the berry extracts except strawberries and blueberries completely inhibited LDL oxidation. Order of the antioxidant activity remained the same with some changes in the levels of inhibition of hexanal formation in the berry extracts pretreated with pectinase (Table 2). The berry extracts were less active antioxidants than catechin (93.4% inhibition), used as a standard compound at 10 μM.

On the basis of the liposome oxidation system, the antioxidant activities of the berries showed different trends compared to the LDL system. At 10 μM GAE, berry extracts inhibited hydroperoxide formation by 27.4−68.8% and hexanal formation by 60.5−92.6%. At 10 μM GAE, the inhibition of hydroperoxides decreased in the following order: sweet cherries (v. Bing) > sweet cherries (v. Burlat) > red raspberries > blackberries > blueberries > strawberries (Table 1). Inhibition of hexanal decreased in the following order: sweet cherries (v. Bing) > sweet cherries (v. Burlat) > blueberries > red raspberries > blackberries > strawberries. At 20 μM GAE, the activity of red raspberries in inhibiting hydroperoxide formation did not increase, but the activity of strawberries in inhibiting hexanal formation improved significantly compared to the other berries. As with the LDL oxidation, the order of the antioxidant activity remained the same in the berry extracts pretreated with pectinase (Table 2). Strawberry extracts pretreated with pectinase (1.0%) inhibited hexanal formation significantly less and blueberry extracts pretreated with pectinase more than acetone extracts. Ascorbic acid either had no effect or was a prooxidant in the liposome system.

Total Phenolics and Phenolic Composition. The berries were extracted with either 70% acetone or 60% methanol, in the presence or absence of pectinase. The amount of total phenolics in the fresh berries varied between 617 and 4350 mg GAE/kg, with the highest amount found in blackberries and the lowest amount in the Burlat variety of sweet cherries (Table 3). With acetone extraction, the yield of total phenolics was always higher although the difference varied between different berries. Also the yields of benzoates, hydroxycinnamates, flavan-3-ols, flavonols, and anthocyanins were usually the highest with acetone extraction. In strawberries the yield of flavan-3-ols and in both Burlat variety of sweet cherries and blackberries the yield of

Table 3. Total Phenolics as GAE (mg/kg) and Classes of Phenolic Compounds (mg/kg) in Berries Extracted with 70% Acetone or 60% Methanol

berry	extraction	total phenolics	benzoates (as gallic acid)	hydroxycinnamates (as caffeic acid)	flavan-3-ols (as catechin)	flavonols (as rutin)	anthocyanins (as malvin)
blackberry	70% Ac	4350 ± 215		15 ± 0.6	108 ± 6	83 ± 0.1	7650 ± 41
	60% MeOH	3610 ± 83		14 ± 0.6	96 ± 5	87 ± 0.0	7430 ± 40
blueberry	70% Ac	3480 ± 343		316 ± 0.9	70 ± 3	139 ± 0.2	4840 ± 28
	60% MeOH	2700 ± 63		226 ± 0.8	63 ± 3	115 ± 0.1	3970 ± 24
red raspberry	70% Ac	3030 ± 75		3.0 ± 0.6	480 ± 37	19 ± 0.3	2200 ± 16
	60% MeOH	2650 ± 295		2.9 ± 0.6	470 ± 36	20 ± 0.3	2210 ± 16
strawberry	70% Ac	2940 ± 300	2.1 ± 1.6	27 ± 0.6	126 ± 8	78 ± 0.1	786 ± 10
	60% MeOH	1610 ± 112		19 ± 0.6	184 ± 11.3	6.1 ± 0.3	768 ± 10
sweet cherry, Bing	70% Ac	805 ± 70	2.5 ± 1.3	133 ± 0.7		18 ± 0.2	40 ± 6
	60% MeOH	784 ± 60	2.3 ± 1.6	115 ± 0.7		10 ± 0.7	31 ± 6
sweet cherry, Burlat	70% Ac	839 ± 70		233 ± 0.6	25.2 ± 0.0	15 ± 0.2	146 ± 7
	60% MeOH	788 ± 13		234 ± 0.6	26.3 ± 0.5	23 ± 0.2	96 ± 7

Table 4. Total Phenolics as GAE (mg/kg) and Classes of Phenolic Compounds (mg/kg) in Blueberries, Strawberries, and Cherries Extracted with 70% Acetone or 60% Methanol with Pectinase Pretreatment

berry	extraction	total phenolics	benzoates (as gallic acid)	hydroxycinnamates (as caffeic acid)	flavan-3-ols (as catechin)	flavonols (as rutin)	anthocyanins (as malvin)
blueberry	70% Ac+p[a]	2720 ± 95	4.7 ± 1.5	156 ± 0.8	18 ± 1	129 ± 0.1	2220 ± 16
	60% MeOH+p	2800 ± 65		251 ± 0.8		194 ± 0.4	2990 ± 25
strawberry	70% Ac+p	2880 ± 11	1.9 ± 1.6	25 ± 0.6	165 ± 1	74 ± 0.1	829 ± 10
	70% Ac+0.5p[b]	2900 ± 12		22 ± 0.6	180 ± 12	25 ± 0.2	840 ± 10
	60% MeOH+p	2640 ± 68	1.8 ± 1.6	21 ± 0.6	169 ± 12	28 ± 0.2	844 ± 10
	60% MeOH+0.5p	2540 ± 35	2.9 ± 1.6	21 ± 0.6	180 ± 12	29 ± 0.2	820 ± 10
sweet cherry, Bing	70% Ac+p	725 ± 45		95 ± 0.7		1.4 ± 0.3	
	60% MeOH+p	753 ± 57		101 ± 0.7			
sweet cherry, Burlat	70% Ac+p	636 ± 3		182 ± 0.6		7.8 ± 0.2	
	60% MeOH+p	617 ± 25		171 ± 0.6		9.5 ± 0.2	

[a] Extraction after treatment with 1.0% pectinase. [b] Extraction after treatment with 0.5% pectinase.

flavonols was slightly higher in the methanolic extract compared to in the acetone extract.

Pectinase is a commonly used enzyme in the maceration of berries in the juice-making process. Treatment of the mash with enzymes is expected to increase yield, reduce processing time, and improve the extraction of important or valued components of the fruit (McLellan, 1996). Pectolytic enzyme preparations can also destroy anthocyanins in raspberry, strawberry, and cherry juices (Jiang et al., 1990). In this study, use of pectinase not only resulted in lower yields of phenolic compounds but also caused losses of flavan-3-ols and anthocyanins in sweet cherries and blueberries (Tables 3 and 4). More hydroxycinnamates and flavonols in blueberries and more flavonols and anthocyanins in strawberries were extracted with methanol after an enzyme treatment compared to methanolic extraction alone. In strawberries rutin was cleaved to quercetin suggesting that the enzyme contained β-glucosidase activity. The concentration of pectinase used in relation to the amount of pectin in the berries is also of importance. Pectinase treatment could be better applied to strawberries where the content of soluble fiber including pectin is higher compared to that in the other berries (Rastas et al., 1993). Treatment with less pectinase (0.5%) resulted in higher yields of strawberry flavan-3-ols compared to acetone extract alone. Moreover, pectinase improved the yield of strawberry anthocyanins. Extraction of strawberries with methanol yielded extracts with a high pectin content making them difficult to handle, concentrate, and filter for HPLC analysis, as reported previously by Gil et al. (1997).

Small amounts of benzoates (1.8−4.7 mg/kg) were identified in blueberries, strawberries, and sweet cherries (v. Bing) but were not found in the extracts of blackberries, red raspberries, and sweet cherries (v. Burlat). Blueberries contained up to 316 mg/kg and sweet cherries up to 234 mg/kg hydroxycinnamates. As determined from the spectral evaluation of the HPLC data, chlorogenic acid (5′-caffeoylquinic acid) predominated in blueberries, as reported previously (Stöhr and Herrman, 1975; Gao and Mazza, 1994), while neochlorogenic acid (3′-caffeoylquinic acid) was present in blackberries. In the early variety of sweet cherries (v. Burlat) 3′-*p*-coumaroylquinic acid accounted for 75% and neochlorogenic acid for 16.5% of the hydroxycinnamates. In the sweet cherries v. Bing the total amount of hydroxycinnamates was approximately one-half that of the early variety with 60% neochlorogenic acid and 40% 3′-*p*-coumaroylquinic acid. Red raspberries contained approximately 4 times more flavan-3-ols (up to 480 mg/kg), tentatively identified as procyanidins and epicatechin, than the other berries. This high content of flavan-3-ols could be due to the presence of seeds housing the flavan-3-ols, as also observed with other seed-containing berries such as strawberries and blackberries. Significant amounts of flavonols such as quercetin glucosides (up to 194 mg/kg) were present in blueberries, blackberries, and strawberries. The predominant group of phenolic compounds in all berries but sweet cherries was anthocyanins, with the highest amount found in blackberries (7650 mg/kg). The amounts of anthocyanins in blackberries and blueberries exceeded that of the measured amount of total phenolics, due to the poor response of anthocyanins in the Folin−Ciocalteu's assay used for analysis of total phenolics. According to Singleton (1974) the response of anthocyanins in the Folin−Ciocalteu's assay is only 0.40 compared to the 1.00 and 0.99 responses of gallic acid and catechin, respectively. Ascorbic acid was only detected in strawberries (590 mg/kg).

Antioxidant Activity vs Phenolic Composition. In the LDL oxidation, the antioxidant activity of berry extracts at 10 μM GAE was related to the presence of

anthocyanins (correlation coefficient, $r = 0.45$) and the absence of flavonols ($r = -0.60$), but not with flavan-3-ols or hydroxycinnamates. In the liposome oxidation model, the antioxidant activity of the berry extracts correlated with the amount of hydroxycinnamates ($r = 0.71$ for hydroperoxide and 0.74 for hexanal formation) and was inversely associated with anthocyanins ($r = -0.47$ and -0.39) and flavonols ($r = -0.42$ for hydroperoxides). The amount of flavan-3-ols did not correlate with the antioxidant activity of berries in the liposome system.

DISCUSSION

All berries reported in this paper inhibited both LDL and liposome oxidation. However, the relative antioxidant activity of berry extracts was different as determined by the two oxidation model systems used. In the LDL oxidation, extracts of blackberries (10 μM GAE) were significantly more active than those of red raspberries, sweet cherries, or blueberries. In the liposome oxidation, extracts of sweet cherries (10 μM GAE) were more active compared to those of other berries. In both oxidation systems strawberries exerted the weakest antioxidant activity. To explain the differences in antioxidant activity of berries when tested under different conditions, differences in the activities of the phenolic compounds and their antagonistic and synergistic reactions with phenolics and other compounds present in berries may be of importance. However, at present, the interpretation is hampered by the fact that while phenolic compounds exist in berries principally either bound to sugars or as esters (Macheix et al., 1990), most of the phenolic antioxidants were tested as their free forms.

Our compositional data on berry phenolics (Table 3) is generally in agreement with earlier reports (Stöhr and Herrmann, 1975, 1976; Möller and Herrmann, 1983; Herrmann, 1989; Costantino et al., 1992; Hertog et al., 1992; Gao and Mazza, 1994, 1995). Acetone was superior to methanol in extracting phenolic compounds from the berry material. Pretreatment with pectinase was applicable for extraction of phenolic compounds in strawberries, but not with the other berries. Except for sweet cherries, the berries were rich in anthocyanins. However, use of acidified solvents (Gao and Mazza, 1995) with the extraction of cherry phenolics most likely would have resulted in higher yields of anthocyanins.

In this study, the presence of anthocyanins and the absence of flavonols in the berries were the best correlated with antioxidant activity toward LDL oxidation. Pure flavonols, such as quercetin, quercetin 3-rutinoside (rutin), and myricetin, almost completely inhibited LDL oxidation when tested at 7.5 and 10 μM (Teissedre et al., 1996; Meyer et al., 1998). However, in this study the flavonol content does not account for the antioxidant activity of berries. Although the most active blackberries contained relatively high amounts of flavonols, the amount of flavonols was 4 times less in the next active red raspberries. In addition, there is no antioxidative synergism between quercetin and cyanidin or quercetin and caffeic acid in the LDL system (Meyer et al., 1998).

Aglycones of anthocyanins were shown to effectively inhibit LDL oxidation (Tamura and Yamagami, 1994; Teissedre et al., 1996; Wang et al., 1997; Satué-Gracia et al., 1997). In red wine, the anthocyanin fraction was the most effective in inhibiting LDL oxidation (Ghiselli et al., 1998). Also the antioxidant activity of grape (Concord) juice samples in preventing LDL oxidation was related to their anthocyanin levels (Frankel et al., 1998). By the same LDL oxidation assay used in the present study, the order of antioxidant activity decreased in the order: delphinidin > cyanidin > malvidin > pelargonidin (Satué-Gracia et al., 1997). This order is consistent with the high antioxidant activity toward LDL for blackberries, which contain the highest amount of cyanidin glycosides (Macheix et al., 1990). The order is also consistent with the low antioxidant activity of strawberries, which are rich in pelargonidin 3-glucoside (Gil et al., 1997), and ascorbic acid, which was also a weak antioxidant in the LDL system.

In the liposome oxidation system, hydroxycinnamic acids present in berries were shown to be the best predictors of the antioxidant activity of berry phenolics. Anthocyanins, flavan-3-ols, and hydroxycinnamic acids were shown to inhibit liposome oxidation (Huang and Frankel, 1997; Satué-Gracia et al., 1997; Heinonen et al., 1998b). The activity of anthocyanins in liposome oxidation is different from that in LDL oxidation. Thus, malvidin is an active antioxidant, whereas delphinidin, cyanidin, and pelargonidin show prooxidant activity (Satué-Gracia et al., 1997). These results may explain why extracts of sweet cherries, which are low in anthocyanins and high in hydroxycinnamates, were the most active in the liposome system compared to blueberries, which are high in both anthocyanins and hydroxycinnamates.

According to Costantino et al. (1992), the activities of black raspberries, black currants, highbush blueberries, blackberries, red currants, and red raspberries toward chemically generated superoxide radicals were greater than those expected on the basis of anthocyanins (200−7000 mg/kg) and polyphenols (2500−12100 mg/kg) present in the berries. Compared to pure delphinidin 3-chloride 8 times less black raspberry or black currant extract was required to cause a 50% inhibition of nitroblue tetrazolium reduction.

To better understand the antioxidant activity of berries, additional data are needed on the activity of phenolic compounds as they occur naturally in bound forms and on their interactions with other compounds present in berries. The present study confirms that berries contribute a significant source of phenolic antioxidants that may have potential health effects.

LITERATURE CITED

Abu-Amsha, R.; Croft, K. D.; Puddey, I. B.; Proudfoot, J. M.; Beilin, L. J. Phenolic content of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation *in vitro*: identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clin. Sci.* **1996**, *91*, 449−458.

Costantino, L.; Albasino, A.; Rastelli, G.; Benvenuti, S. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Medica* **1992**, *58*, 342−344.

Criqui, M. H.; Ringel, B. L. Does diet or alcohol explain the French paradox? *Lancet* **1994**, *344*, 1719−1723.

Frankel, E. N.; German, J. B.; Davis, P. A. Headspace gas chromatography to determine human low-density lipoprotein oxidation. *Lipids* **1992**, *27*, 1047−1051.

Frankel, E. N.; Bosanek, C. A.; Meyer, A. M.; Silliman, K.; Kirk, L. L. Commercial grape juices inhibit the in vitro oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1998**, *46*, 834−838.

Gao, L.; Mazza, G. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J. Food Sci.* **1994**, *59*, 1057−1059.

Gao, L.; Mazza, G. Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *J. Agric. Food Chem.* **1995**, *43*, 343−346.

Ghiselli, A.; Nardini, M.; Baldi, A.; Scaccini, C. Antioxidant activity of different phenolic fractions separated from Italian red wine. *J. Agric. Food Chem.* **1998**, *46*, 361−367.

Gil, M. I.; Holcroft, D. M.; Kader, A. A. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem.* **1997**, *45*, 1662−1667.

Heinonen, I. M.; Lehtonen, P. J.; Hopia, A. I. Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem.* **1998a**, *46*, 25−31.

Heinonen, I. M.; Rein, D.; Satué-Gracia, T. M.; Huang, S.-W.; German, J. B.; Frankel, E. N. Effect of protein on the antioxidant activity of phenolic compounds in a lecithin-liposome oxidation system. *J. Agric. Food Chem.* **1998b**, *46*, 917−922.

Herrmann, K. Occurrence and content of hydroxycinnamates and hydroxybenzoic acid compounds in foods. *CRC Rev. Food Sci. Nutr.* **1989**, *28*, 315−347.

Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem.* **1992**, *40*, 2379−2383.

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.

Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and cancer risk in the Zutphen elderly study. *Nutr. Cancer* **1994**, *22*, 175−184.

Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, 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.

Huang, S.-W.; Frankel, E. N. Antioxidant activity of tea catechins in different lipid systems. *J. Agric. Food Chem.* **1997**, *45*, 3033−3038.

Jiang, J.; Paterson, A.; Piggott, J. R. Short communication: effects of pectolytic enzyme treatments on anthocyanins in raspberry juice. *Int. J. Food Sci. Technol.* **1990**, *25*, 596−600.

Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. Natural antioxidants in grapes and wines. *J. Agric. Food Chem.* **1994**, *42*, 64−69.

Knekt, P.; Järvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med J.* **1996**, *312*, 478−481.

Knekt, P.; Järvinen, R.; Seppänen, R.; Heliövaara, M.; Teppo, L.; Pukkala, E.; Aromaa, A. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am. J. Epidemiol.* **1997**, *146*, 223−230.

Lamuela-Raventos, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994**, *45*, 1−5.

Lanningham-Foster, L.; Chen, C.; Chance, D. S.; Loo, G. Grape extract inhibits lipid peroxidation of human low-density lipoprotein. *Biol. Pharm. Bull.* **1995**, *18*, 1347−1351.

Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990.

McLellan, M. R. Juice Processing. In *Processing Fruits: Science and Technology. Volume 1. Biology, Principles, and Applications*; Somogyi, L. P., Ramaswamy, H. S., Hui, Y. H., Eds.; Technomic Publishing Co. Inc.: Lancaster, 1996; pp 67−94.

Meyer, A. S.; Yi, O.-S.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **1997**, *45*, 1638−1643.

Meyer, A. S.; Heinonen, M.; Frankel, E. N. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food Chem.* **1998**, *61*, 71−75.

Miller, N. J.; Diplock, A. T.; Rice-Evans, C. A. Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *J. Agric. Food Chem.* **1995**, *43*, 1794−1801.

Möller, B.; Herrmann, K. Quinic acid esters of hydroxycinnamic acids in stone and pome fruit. *Phytochemistry* **1983**, *22*, 477−481.

Orr, J. R.; Adamson, G. L.; Lindgren, F. T. Preparative ultracentrifugation and analytic ultracentrifugation of plasma lipoproteins. In *Analysis of Fats, Oils and Lipoproteins*; Perkins, E. G., Ed.; American Oil Chemists' Society: Champaign, IL, 1991; pp 524−554.

Pilando, L. S.; Wrolstad, R. E.; Heatherbell, D. A. Influence of fruit composition, maturity, and mold contamination on the color and appearance of strawberry wine. *J. Food Sci.* **1985**, *50*, 1121−1125.

Plumb, G. W.; Chambers, S. J.; Lambert, N.; Bartolomé, B.; Heaney, R. K.; Wanigatunga, S.; Aruoma, O. I.; Halliwell, B.; Williamson, G. Antioxidant actions of fruit, herb and spice extracts. *J. Food Lipids* **1996**, *3*, 171−188.

Rastas, M.; Seppänen, R.; Knuts, L.-R.; Karvetti, R.-L.; Varo, P. *Nutrient Composition of Foods*; Publications of the Social Insurance Institution: Finland, 1993.

Satué-Gracia, M. T.; Heinonen, I. M.; Frankel, E. N. Anthocyanins as antioxidants on human low-density lipoprotein and lecithin-liposome systems. *J. Agric. Food Chem.* **1997**, *45*, 3362−3367.

Singleton, V. L. Analytical fractionation of the phenolic substances of grapes and wine and some practical use of such analyses. In *Chemistry of Winemaking*; Webb, A. D., Ed.; American Chemical Society: Washington, DC, 1974.

Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Contr.* **1991**, *2*, 325−357.

Starke, H.; Herrmann, K. Changes in flavonol concentrations during fruit development. *Z. Lebensm. Unters.-Forsch.* **1976**, *161*, 131−135.

Stöhr, H.; Herrmann, K. The phenolics of fruits. VI. The phenolics of currants, gooseberries and blueberries. Changes in phenolic acids and catechins during development of black currants. *Z. Lebensm. Unters.-Forsch.* **1975**, *159*, 341−347.

Tamura, H.; Yamagami, A. Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. *J. Agric. Food Chem.* **1994**, *42*, 1612−1615.

Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701−705.

Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 304−309.

Received for review February 25, 1998. Revised manuscript received July 29, 1998. Accepted July 30, 1998. The support from Yrjö Jahnsson, Ella and Georg Erhnroot, Food Research, and Juho Vainio Foundations is gratefully acknowledged.

JF980181C