# **Antioxidant Activity of Berry and Fruit Wines and Liquors**

I. Marina Heinonen,\*,† Pekka J. Lehtonen,‡ and Anu I. Hopia†

Department of Applied Chemistry and Microbiology, P.O. Box 27, Food Chemistry, FIN-00014 University of Helsinki, Finland, and Alcohol Control Laboratory, Alko Group Inc., P.O. Box 350, FIN-00101 Helsinki, Finland

A total of 44 different berry and fruit wines and liquors with total phenolic contents between 91 and 1820 mg/L, expressed as gallic acid equivalents (GAE), were evaluated for antioxidant activity. Dealcoholized wine extracts were added to methyl linoleate (MeLo), and the oxidation in the dark at 40 °C was followed by conjugated diene measurement. Wines made of mixtures of black currants and crowberries or bilberries (240–275  $\mu$ M GAE) were slightly superior to reference red grape wines (330–375  $\mu$ M GAE) and equally as active as the control antioxidant,  $\alpha$ -tocopherol (50  $\mu$ M), in inhibiting MeLo hydroperoxide formation. Also, raw materials including apple, arctic bramble, cowberries, cranberries, red currants, or rowanberries possessed antioxidant activity. Thus, these raw materials contain phenolic compounds, some of which are capable of protecting lipids against oxidation also in a hydrophobic lipid system. Liquors, apart from arctic bramble liquor, were less active than wines. However, the total phenolic content did not correlate with the antioxidant activity of the berry and fruit wines and liquors, therefore alleviating the importance of further characterization of the phenolic antioxidants present in berry and fruit wines.

Keywords: Berry and fruit wines and liquors; antioxidant activity; total phenolics; methyl linoleate

## INTRODUCTION

Natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects. Because extensive and expensive testing of food additives is required to meet safety standards, synthetic antioxidants have generally been eliminated from many food applications (Frankel, 1995). The increasing interest in the search for natural replacements for synthetic antioxidants has led to the antioxidant evaluation of a number of plant sources. The most current research on antioxidant action focuses on phenolic compounds such as flavonoids. Phenolic compounds in red grape wine have been shown to inhibit in vitro oxidation of human low-density lipoprotein (LDL) (Frankel et al., 1993, 1995; Kinsella et al., 1993; Kanner et al., 1994; Teissedre et al., 1996; Abu-Amsha et al., 1996). Moreover, dietary intake of flavonoids has been shown to be inversely related to coronary heart disease mortality (Hertog et al., 1993, 1995; Knekt et al., 1996).

For the production of berry and fruit wines, pressed juice is made from the berries and fruits such as apples, pears, cherries, plums, peaches, red currants, gooseberries, bilberries, cranberries, raspberries, hip berries, and rhubarb (Belitz and Grosch, 1987). In general, the berry and fruit wine-making process is the same as making wine from grapes; that is, the berry or fruit mash is first pressed, and then the pressed juice is fermented. Fruits and berries used for production of liquors include apricot, arctic bramble, cloudberry, peach, bilberry,

raspberry, strawberry, and red currant. Berry and fruit wines and liquors are produced industrially in many countries, e.g. apple wine (cider) in France, the United Kingdom, and the United States and pear wine known as "poiré" in France (Belitz and Grosch, 1987). Other berry and fruit wines are produced mainly for domestic use in some European Union countries such as Germany, Sweden, and Finland.

Berries and fruits contain a wide range of flavonoids and other phenolic compounds that possess antioxidant activity. Most of these compounds remain present also in berry and fruit products, such as juices. However, losses of anthocyanins in juices and purées of strawberries (Bakker and Bridle, 1992), strawberry and black currant syrups (Skrede et al., 1992), and raspberry juice and wine (Rommel et al., 1990) have been reported as well as phenolic degradation during processing of apple juice (Spanos et al., 1990). On the other hand, during the grape wine-making process significant changes in phenolic composition of the raw material occur both very early at the grape crushing step and during wine fermentation and aging (Singleton, 1987). Therefore, it is most likely also that during berry and fruit wine processing phenolic compounds undergo changes and are effectively extracted into berry and fruit wines.

Very scarce data on the antioxidant activity of berries and fruits and their products, apart from grape wine, in various oxidation models have been reported. In an artificial peroxyl radical model system fresh strawberry was reported to have 3.5 times higher total antioxidant capacity than apple and 15 times higher than trolox, the water soluble analogue of  $\alpha$ -tocopherol (Wang et al., 1996). The antioxidant activity of grapes in inhibiting oxidation of human LDL has been shown comparable to that of wine (Meyer et al., 1997). Among commercial fruit juices, grape juice has been demonstrated to have the highest antioxidant capacity followed by grapefruit

<sup>\*</sup> Author to whom correspondence should be addressed [fax (+358) 9 708 5475; e-mail marina.heinonen@helsinki.fi].

<sup>&</sup>lt;sup>†</sup> Department of Applied Chemistry and Microbiology.

<sup>‡</sup> Alcohol Control Laboratory.

juice, tomato juice, orange juice, and apple juice (Wang et al., 1996). Moreover, grape juice has been shown to inhibit oxidation in human LDL (Lanningham-Foster et al., 1995; Abu-Amsha et al., 1996) and in a  $\beta$ -carotene—linoleate system (Kanner et al., 1994). In a trolox equivalent antioxidant capacity assay, apple juice with or without added ascorbic acid exerted antioxidant activity, most of the antioxidant capacity being due to chlorogenic acid (32%) or added ascorbic acid (94%) (Miller et al., 1995). The phenolics in fruit juices have an ascorbate-sparing effect in the order of black currant > orange > apple (Miller and Rice-Evans, 1997). However, data on antioxidant activity of berry and fruit wine phenolics have not been previously reported.

The purpose of this study was to evaluate the antioxidant activity of 44 berry and fruit wines and liquors on oxidation of methyl linoleate as part of a collaborative attempt to optimize utilization of naturally occurring antioxidants from plant foods.

### MATERIALS AND METHODS

Berry and Fruit Wines and Liquor Extracts. A total of 33 different berry and fruit wines and 11 liquors were kindly given by commercial and local producers. The raw materials of the berry and fruit wines included apple, arctic bramble, aronia, bilberry, cherry, cloudberry, cowberry, cranberry, crowberry, black, red, and green currant, honey, red raspberry, rhubarb, rowanberry, and strawberry (Table 1). The wines were dealcoholized by adding 10 mL of H2O to 10 mL of wine, and the resulting mixture was evaporated to 10 mL using rotary evaporator. The pH was adjusted to 7.0 using 5 M NaOH. Solid-phase extraction (SPE) was used to remove sugars in the wines. SPE tubes (C<sub>18</sub> Sep-Pak Vac 200 mg, Millipore Waters, Milford, MA) were preconditioned by washing them with 2.5 mL of methanol and with 5 mL of  $H_2O$ . Prepared wine (0.5 mL) was transferred to the SPE tube, and the tube was washed with 2 mL of H<sub>2</sub>O (pH 2.0) prior to elution of the wine fraction with 2.5 mL of methanol. The methanol fraction was taken to dryness under N2 and the solid remainder was stored in a freezer until the antioxidant studies were performed. The sugar removal procedure was validated with samples 2, 3, 5, 8, 11, 12, 16, 19, 20, 26, 29, 31, and 39 with sugar content varying between <9 and 320 g/L.

**Chemicals.** Methyl linoleate (MeLo) was purchased from Nu-Check-Prep, Inc. (Elysian, MN), and  $\alpha$ -tocopherol, gallic acid, and Folin–Ciocalteu's reagent were purchased from Sigma (St. Louis, MO). All organic solvents used were of HPLC grade.

**Determination of Total Phenolics.** The amount of total phenolics in berry and fruit wines before and after removal of sugars was determined according to the Folin–Ciocalteu procedure (Folin-Ciocalteu Index, 1990) and expressed as milligrams per liter gallic acid equivalents (GAE).

Oxidation of MeLo. The method used has been applied to antioxidant activity studies of carnosol and carnosic acid (Hopia et al., 1996) and to  $\alpha$ -tocopherol (Huang et al., 1996). Reconstituted methanolic berry and fruit wine and liquor extracts (50 and 200  $\mu$ L) were added to MeLo (1 g), and methanol was evaporated under nitrogen. Oxidation of MeLo was carried out in the dark at 40 °C. Sample aliquots (10 mg) were taken at regular intervals and dissolved in 5 mL of 2,2,4trimethylpentane (isooctane) for spectrophotometric measurements (Perkin-Elmer lamda 15 UV-vis spectrophotometer, Norwalk, CT) of conjugated diene absorption at 234 nm. The spectrophotometer was set to zero with isooctane. The amount of hydroperoxides was calculated using absorptivity of 26 000 (Fishwick and Swoboda, 1977). The antioxidant activity was expressed as percentual (%) inhibition of formation of MeLo hydroperoxides calculated at the times when the amount of hydroperoxides had reached 400 and 800 mmol/kg of lipid in the control sample. One European white grape wine (A) and three European red grape wines (B-D) treated as the berry

and fruit wines were used as reference wines. Moreover, 50  $\mu M$   $\alpha\text{-tocopherol},$  a known lipid antioxidant, was used in each experiment as a control antioxidant.

## **RESULTS**

**Amount of Total Phenolics.** The amount of total phenolics in the berry and fruit wines and liquors ranged from 91 to 1820 mg/L GAE (Table 1). Wines made of cherries (1080 mg/L GAE), red raspberries and black currants (1050 mg/L GAE), black currants and bilberries (average 1040 mg/L GAE), black currants and crowberries (1020 mg/L GAE), black and red currants (average 890 mg/L GAE), and black currants (average 870 mg/L GAE) contained the highest amounts of phenolic compounds. Marked amounts of total phenolics were also present in wines made of cowberries and birch sap (776 mg/L GAE) as well as in wines made of mixtures of black and/or red currants and strawberry (average 755 mg/L GAE). In red grape wines used as reference wines, the amount of total phenolics ranged from 1390 to 1500 mg/L GAE. Honey, rhubarb, and green and white currant wines contained very low amounts of phenolic compounds: 91, 125, and 260 mg/L GAE, respectively. Also, the reference white grape wine contained a low amount of phenolic compounds: 265 mg/L GAE. The removal of sugars prior to the antioxidant activity testing reduced the amount of total phenols to 80  $\pm$  5%. However, removal of sugars was a necessary step to prevent interference during oxidation of MeLo at 40 °C.

**Antioxidant Activity of Wines and Liquors.** The wines made of mixtures of black currants and bilberries and of black and red currants showed the highest antioxidant activity. The average inhibition of oxidation of these berry and fruit wines at concentrations of 129-300  $\mu M$  GAE ranged from 60.5 to 93.5% measured at the time when the amount of hydroperoxides in the control had reached 800 mmol/kg MeLo (Table 1). Also, single wines made of mixtures of black currants and crowberries (98% inhibition), cranberries (92%), rowanberries and apple (90%), apples (84%), and cowberries and birch sap (69%) as well as light liquor of arctic bramble (78%) were efficient as antioxidants. The inhibition of MeLo oxidation with reference red wines  $(330-375 \mu M GAE)$  ranged from 93 to 97%. Wines made of mixtures of black currants and crowberries or bilberries (240–275  $\mu$ M GAE) were superior to other berry and fruit wines as well as to the reference red wines and equally as active as  $\alpha$ -tocopherol (50  $\mu$ M) in inhibiting hydroperoxide formation. The antioxidant activity of liquors, apart from arctic bramble liquor, was generally lower than that of wines. The reference white wine as well as berry and fruit wines and liquors made of honey, cloudberries, mixtures of cloudberries and red raspberries, rowanberries, and strawberries did not possess antioxidant activity. Moreover, honey and cloudberry wines exerted prooxidant effects. Inhibition of MeLo oxidation of selected berry wines and liquors is shown in Figures 1 and 2.

Total Phenolic Content vs Antioxidant Activity. There was no correlation between the total phenolic content and the antioxidant activity of the berry and fruit wines. The correlation coefficient, r, was 0.30 (Figure 3), 0.35, 0.38, and 0.38 when the actual phenolic content was correlated with inhibition of oxidation of 50 and 200  $\mu$ L of wine extract at a control level of 400 mmol of hydroperoxides/kg of MeLo and of 50 and 200

Table 1. Total Phenolics in Berry and Fruit Wines and Their Inhibition (% In)<sup>a</sup> of Methyl Linoleate Oxidation

		alaal: J	total phenolics,		% In,	% In,	% In,	% In,
raw materials of berry and		alcohol, vol %	mg of GAE/L	$\mu$ M GAE in 50 $\mu$ L <sup>c</sup>	50 μL (DV 400)	200 μL (DV 400)	50 μL,	200 μL
fruit wines and liquors	g/L	V01 %	(after SPE) <sup>b</sup>	III συ μL°	(PV 400)	(PV 400)	(PV 800)	(PV 800
wines	_							
1 black currant and crowberry (3/1)	<9	12	1020 (820)	240	97	97	98	99
2 black currant and bilberry	15	13	1010 (850)	250	96	96	96	97
3 black currant and bilberry (2/1)	<9	13.5	1060 (940)	275	95	96	91	97
4 black and red currant	<45	12	1035 (830)	245	97	97	98	99
5 black and red currant (7/1)	<9	13	980 (780)	230	95	98	81	79
6 black and red currant	28	11	680 (540)	160	94	96	69	96
7 black and red currant (3/4)	30-40	13	515 (440)	130	80	95	48	85
8 black and red currant (2/1)	44	12	870 (700)	205	89	96	34	75 50
9 black and red currant	15	13	1270 (1020)	300	55	88	33	58
0 black currant	<9	12	520 (410)	120	93	98	65	98
1 black current	<9	12	1820 (830)	245	93	96	64	96
2 black currant	28	12	995 (800)	235	69	91	28	51
3 black currant	< 45	5.5	870 (700)	205	37	66	19	13
4 black currant	100	12 12	1000 (750)	880	8	95 93	5	91 70
5 black and red currant and strawberry	20	12	720 (575)	170	70	93	41	70
(1/1/1) 6 black currant and strawberry	35	12	605 (550)	160	93	95	65	90
7 black currant and strawberry	<9	12	695 (550)		93 60	95 46	28	90 19
			655 (525)	155				19 97
8 black currant and strawberry (4/1) 9 red currant	43 30-50	13 12	950 (760)	225 1000	95 80	96 96	8 41	97 96
9 red currant 0 red currant	30-30 100	16	495 (340) 440 (340)	1000	68	80	39	90 44
_	>45	13	470 (375)	110	93	94	39 84	90
1 apple 2 apple	< 45	12	160 (130)	38	59	81	40	42
	<45	12	240 (190)	56	70	90	36	61
3 apple 4 apple	<45	12	260 (210)	62	70 59	86	22	53
	< <b>20</b>	13	690 (550)	160	76	48	90	93
5 rowanberry and apple 6 cowberry and birch sap	110	10	775 (600)	175	70 88	96	69	96
7 cranberry	<45	12	680 (540)	160	75	20	92	52
8 green and white currant	120	11	250 (200)	59	83	90	52 59	65
9 green and white currant (1/4)	< 45	12	270 (220)	65	53	90	25	64
green and white currants	<20	12	265 (210)	62	34	81	14	51
1 strawberry and black currant	40	13.5	335 (250)	73	5 <del>1</del>	79	41	65
2 rhubarb	40	13.3	125 (100)	73 29	81	65	3	8
3 honey	<9	4.5	91 (73)	21	0	-10	-3	0
3 noney	`∂	4.5	91 (73)	٤1	U	-10	-3	U
liquors								
4 cranberry	300	21	500 (400)	120	15	45	3	10
5 cherry	180	21	1080 (865)	255	10	80	30	53
6 arctic bramble	140	15	555 (445)	130	24	81	78	78
7 arctic bramble	480	27.5	610 (490)	145	48	63	26	36
8 strawberry	130	15	525 (420)	125	35	53	21	26
9 strawberry	320	21	410 (350)	105	-6	19	3	10
0 rowanberry	>200	21	545 (435)	130	26	93	7	62
1 cloudberry	250	21	500 (400)	120	-30	20	3	10
2 cloudberry	335	21	450 (360)	105	-13	-55	-5	-33
3 cloudberry and red raspberry	300	21	415 (330)	97	-5	15	0	13
4 red raspberry and black currant reference grape wines	>200	21	1050 (840)	245	49	79	12	-2
white wine A	<9	12	265 (212)	78	19	25	4	8
red wine B		12	1600 (1280)	375	$94.7 \pm 1.5$	$97.3 \pm 1.2$	$90.0 \pm 9.6$	$95.0 \; \pm$
red wine C		12	1390 (1100)	330	92	93	95	97
red wine D		12	1500 (1200)	350	93	94	83	98
control			, ,				$94.0 \pm 4.4$	
50 $\mu$ M α-tocopherol					$94.6 \pm 4.6$			

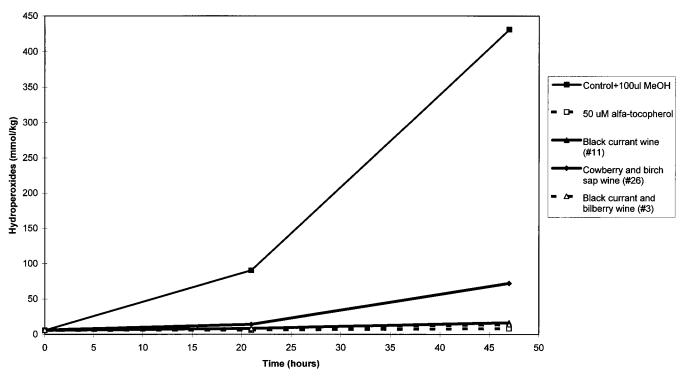
<sup>a</sup> Percentual inhibition of formation of MeLo hydroperoxides measured at the times when the amount of hydroperoxides (PV, peroxide value) in the control sample was 400 or 800 mmol/kg of MeLo. Two concentrations, 50 and 200 µL, of the berry and fruit wine extracts were added to 1 g of MeLo. b Total phenolics expressed as GAE before and after removal of sugars with SPE. The average loss of phenolic compounds in the SPE was  $80 \pm 5\%$ .  $^c\mu M$  ( $\mu mmol/kg$  of MeLo) GAE when 50  $\mu L$  of wine was added to MeLo.

 $\mu L$  of wine extract at a control level of 800 mmol of hydroperoxides/kg of MeLo, respectively. The amount of total phenolics in the wine extracts ranged from 21 to 300  $\mu$ M GAE (50  $\mu$ L of wine added to MeLo) and from 86 to 1200  $\mu$ M GAE (200  $\mu$ L) (Table 1). The correlation was even worse within the berry and fruit wines containing relatively high amounts of total phenolics, >690 mg/L GAE, after SPE. The r values were 0.08, 0.14, 0.30, and 0.18 when the phenolic content (>690 mg/L GAE) was correlated with inhibition of oxidation of 50 and 200  $\mu$ L of wine extract at control level of 400 mmol of hydroperoxides/kg of MeLo and of 50 and 200

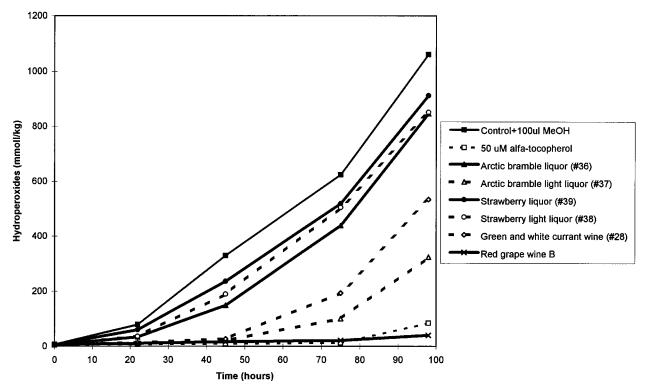
 $\mu L$  of wine extract at a control level of 800 mmol of hydroperoxides/kg of MeLo, respectively.

# DISCUSSION

**Amount of Total Phenolics.** It is evident that berry and fruit wines in general contain lower amounts of phenolic compounds than red grape wines. Red grapes, wines, and grape byproducts reportedly contain high amounts of phenolic compounds, 500-4059 mg/L GAE (Macheix et al., 1990; Kanner et al., 1994; Frankel et al., 1995; Meyer et al., 1997). Only in a very few berry



**Figure 1.** Antioxidant activity of black currant (no. 11), black currant and bilberry (no. 3), and cowberry and birch sap wines (no. 26) (50  $\mu$ L) on oxidation of MeLo at 40 °C.  $\alpha$ -Tocopherol (50  $\mu$ M) was used as a control.



**Figure 2.** Antioxidant activity of arctic bramble liquor (no. 36) and light liquor (no. 37), strawberry liquor (no. 39) and light liquor (no. 38), and green and white currant wine (no. 28) (50  $\mu$ L) on oxidation of MeLo at 40 °C. α-Tocopherol (50  $\mu$ M) and red wine B (50  $\mu$ L) were used as controls.

wines, including wines made of black currants alone or in mixtures with bilberries or red currants, did the total phenolic content exceed 1000 mg/L GAE. For example, black currant wine 11 had an exeptionally high phenolic content of 1820 mg/L GAE compared to the phenolic content of the three red grape wines (1390–1600 mg/L GAE) used as reference wines. High phenolic content in the raw materials or different wine-making techniques such as prolonged extraction time are plausible

explanations for the high phenolic content in black currant wine 11. The amount of black currants used or the skin contact time during the wine-making process did not differ markedly from those used for the other black currant wines tested. On the contrary, prolonged skin contact did not increase the total phenolic content in wines 1 and 3. However, these wines were highly active in retarding oxidation of MeLo, thus suggesting that the prolonged skin contact may have resulted in

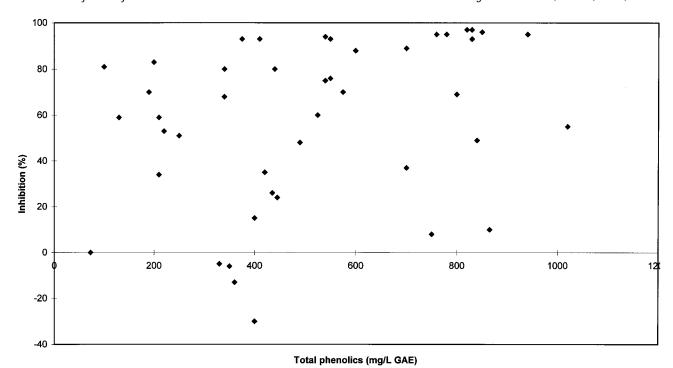


Figure 3. Total phenolic content after SPE vs inhibition (percent) of MeLo oxidation by low concentrations (129–300 µM GAE in 50 µL of wine/g of MeLo) of berry and fruit wines measured at the level of 400 mmol/kg hydroperoxides in the control.

an effective release of the antioxidatively active phenolic compounds from the raw material. In grape wines, especially the concentration of flavan-3-ols such as catechin increases (Alonso et al., 1991; Escribano-Bailón et al., 1995; Meyer et al., 1997) and red pigments, i.e., anthocyanins, are efficiently released (Belitz and Grosch, 1987) with prolonged contact with skin as well as seeds. On the other hand, unprotected anthocyanin pigments have been reported to decrease dramatically, cyanidin 3-glucoside being the most unstable, during fermentation of blackberries and red raspberries into wines (Rommel et al., 1990, 1992). Apart from cyanidin glycosides generally found in all berries and fruits, black currants as well as bilberries contain delphinidin glycosides (Macheix et al., 1990)

**Antioxidant Activity of Wines and Liquors.** To explain the differences in antioxidant activity, factors such as solubilities and partitioning between different phases may also be important. Berry and fruit extracts containing flavonoids and other related phenolic compounds have mainly been reported to show antioxidant activity in water-rich conditions such as in oxidation of human LDL (Frankel et al., 1993, 1995; Kinsella et al., 1993; Kanner et al., 1994; Lanningham-Foster et al., 1995; Teissedre et al., 1996; Abu-Amsha et al., 1996), in a hydrophilic artificial peroxyl radical model system (Wang et al., 1996; Miller and Rice-Evans, 1997), and in an aqueous  $\beta$ -carotene—linoleate system (Kanner et al., 1994). Black currant juice is reported by Miller and Rice-Evans (1997) to have an ascorbate-sparing effect. The present study shows that extracts of dealcoholized berry and fruit wines made of mixtures of black currants with crowberries or bilberries and mixtures of black and red currants as well as wines including raw materials such as apple, arctic bramble, cowberries, cranberries, or rowanberries possess antioxidant activity in a bulk lipid (MeLo) model. Moreover, the antioxidant activities of wines made of mixtures of black currants and crowberries or bilberries were superior to other berry and fruit wines as well as to red wine and equally as

active as  $\alpha$ -tocopherol. These raw materials contain phenolic compounds capable of protecting lipids against oxidation also in hydrophobic lipid systems, thus suggesting potential benefits in attempts to utilize naturally occurring compounds as antioxidants in processed

Antioxidant activity of white wines is reported (Frankel et al., 1995; Abu-Amsha et al., 1996) to be markedly lower compared to the activity of red wines due to their lower content of phenolics. This was confirmed in the present study, in which white wine containing low amount of phenolics, 265 mg/L GAE, had practically no effect on the oxidation of MeLo. Also, most of the fruit and berry wines with a phenolic content <600 mg/L GAE did not markedly inhibit the oxidation of MeLo. The inactive raw materials included cloudberries, red raspberries, and strawberries, all berries being soft with a thin skin as well as light in color. These raw materials being inactive adds to the conclusion that berries with strong color and relatively small size with a higher proportion of tough skin such as bilberries, black currants, cowberries, cranberries, and crowberries exert antioxidant activity.

The very strongly colored berries such as bilberries, black currants, and crowberries are the richest in anthocyanins (Macheix et al., 1990). According to Stöhr and Herrman (1975), black, red, and white currants and cultivated blueberries contain only small amounts of catechins, but hydroxycinnamic acid derivatives occur mostly in higher concentrations. Caffeic acid derivatives are the most abundant hydroxycinnamates, representing 84-94, 17-41, 42-48, 90-100, and 35-87, respectively, in apple, black currant, red currant, blueberry, and sweet cherries (Macheix et al., 1990). Bilberries are especially rich in chlorogenic acid (Stöhr and Herrman, 1975). Hydroxycinnamic acids and their derivatives (Laranjinha et al., 1994; Nardini et al., 1996; Teissedre et al., 1996) as well as anthocyanins (Tamura and Yamagami, 1994; Teissedre et al., 1996; Wang et al., 1997) have been shown to inhibit oxidation in various model systems. Moreover, caffeic acid fractionated from hydrolyzed red wine has been shown to be the most active hydroxycinnamate in inhibiting oxidation of human LDL (Abu-Amsha et al., 1996).

**Total Phenolic Content vs Antioxidant Activity.** The Folin-Ciocalteu assay for total phenolics (Singleton and Rossi, 1965) correlates well with the relative antioxidant activity measured in in vitro LDL oxidation in grape wines (r = 0.94) (Frankel et al., 1995) and in grapes (r = 0.89) (Meyer et al., 1997). On the contrary, in the present study there was no correlation between the total phenolic content and the antioxidant activity of the berry and fruit wines (r values ranged from 0.32 to 0.47) and even less so within the berry and fruit wines containing relatively high amounts of total phenolics. Many fruits and berries contain significant amounts of anthocyanins compared to other flavonoids, markedly less flavan-3-ols, and generally less hydroxycinnamic acids than grapes (Macheix et al., 1990). According to Singleton (1974) anthocyanins respond poorly in the Folin—Ciocalteu assay, their response being 0.40 compared to the 1.00 and 0.99 responses of gallic acid and catechin, respectively. Therefore, the total phenolic content, i.e., the reducing capacity of berry and fruit wines, does not accurately respond to the true antioxidant nature of their phenolic contstituents. Grape wines, on the other hand, are especially rich in gallic acid and catechin, although according to Frankel et al. (1995) the capacity to protect LDL from oxidation appears to be distributed widely among a large number of phenolic constituents in wine. Moreover, the lack of correlation between the total phenolic content and the antioxidant activity of berry and fruit wines may also in part be explained by the wide range of raw materials differing significantly in their composition of phenolic compounds.

The antioxidant activity of apple wines varied from 22 to 84% (average 45.5%) inhibition at the level of hydroperoxides in control 800 mmol/kg lipid with no correlation to the total phenolic content of these wines. According to Miller et al. (1995), in a trolox equivalent antioxidant capacity assay most of the antioxidant capacity is due to added ascorbic acid (94%). In the present study, however, ascorbic acid was removed in the SPE together with the sugars. Thus, it seems that the antioxidant activity of individual phenolic compounds as well as synergistic and antagonistic effects together with other compounds present in the wine determines the antioxidant activity of the berry or fruit wine. However, without compositional analytical data on phenolic compounds in berry and fruit wines the correlation between the antioxidant activities of the wines and their active components cannot be fully described. To clarify the antioxidant potency of berry and fruit wines, detailed analyses and identification of phenolic antioxidants in these wines are currently under way.

## ACKNOWLEDGMENT

We thank Ms. Leena Varo and M.Sc. Urve Jakobson for technical assistance, the berry and fruit wine producers, and the Juho Vainio Foundation for support.

### 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.
- Alonso, E.; Bourzeix, M.; Revilla, E. Suitability of water/ ethanol mixtures for the extraction of catechins and proanthocyanidins from *Vitis vinifera* seeds contained in a winery by-product. *Seed Sci. Technol.* **1991**, *19*, 545–552.
- Bakker, J.; Bridle, P. Strawberry juice colour: the effect of sulphur dioxide and EDTA on the stability of anthocyanins. *J. Sci. Food Agric.* **1992**, *60*, 477–481.
- Belitz, H.-D.; Grosch, W. *Food Chemistry*; Springer-Verlag: Berlin, 1987; p 774.
- Escribano-Bailón, M. T.; Guerra, M. T.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Proanthocyanidins in skins from different grape varieties. *Z. Lebensm. Unters. Forsch.* **1995**, *200*, 221–224.
- Fishwick, M. J.; Swoboda, P. A. T. Measurement of oxidation of polyunsaturated fatty acids by spectophotometric assay of conjugated derivatives. *J. Sci. Food Agric.* **1977**, *28*, 387–393.
- Folin-Ciocalteu Index. Off. J. Eur. Communities 1992, 178–179.
- Frankel, E. N. Natural and biological antioxidants in foods and biological systems. Their mechanisms of action, applications and implications. *Lipid Technol.* **1995**, July, 77–80
- Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454–457.
- Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890–894.
- 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.; 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.
- Hopia, A.; Huang, S.-W.; Schwarz, K.; German, B. J.; Frankel, E. N. Effect of different lipid systems on antioxidant activity of rosemary constituents carnosol and carnosic acid with and without α-tocopherol. *J. Agric. Food Chem.* **1996**, *44*, 2030–2036
- Huang, S.-W.; Hopia, A.; Schwarz, K.; Frankel, E. N.; German, B. J. Antioxidant activity of α-tocopherol and trolox in different lipid substrates: bulk oils vs oil-in-water emulsions. J. Agric. Food Chem. 1996, 44, 444–452.
- 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.
- Kinsella, J. E.; Frankel, E.; German, B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* **1993**, *47*, 85–89.
- 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.
- 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.
- Laranjinha, J. A. N.; Almeida, L. M.; Madeira, V. M. C. Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low-density lipoprotein peroxidation. *Biochem. Pharmacol.* **1994**, *48*, 487–494.
- Macheix, J.-J.; Fleurit, A.; Billot, J. Fruit Phenolics, CRC Press: Boca Raton, FL, 1990.
- 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.
- Miller, N. J.; Rice-Evans, C. A. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chem. 1997, 60, 331-337.
- 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.
- Nardini, M.; D'Aquino, M.; Tomassi, G.; Gentili, V.; De Felice, M.; Scaccini, C. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radical Biol. Med. 1995, 19, 541-552.
- Rommel, A.; Heatherbell, D. A.; Wrolstad, R. E. Red raspberry juice and wine: effect of processing and storage on anthocyanin pigment composition, color and appearance. J. Food *Sci.* **1990**, *55*, 1011–1017.
- Rommel, A.; Wrolstad, R. E.; Heatherbell, D. A. Blackberry juice and wine: processing and storage effects on anthocyanin composition, color and appearance. J. Food Sci. 1992, *57*, 385–410.
- Singleton, V. L. Analytical fractionation of the phenolic substances of grapes and wine and some practical uses of such analyses. In *Chemistry of Winemaking*; Webb, A. D., Ed.; American Chemical Society: Washington, DC, 1974.
- Singleton, V. L. Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69–77.
- Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. **1965**, 16, 144–158.
- Skrede, G.; Wrolstad, R. E.; Lea, P.; Enersen, G. Color stability of strawberry and blackcurrant syrups. J. Food Sci. 1992, *57*, 172–177.

- Spanos, G. A.; Wrolstad, R. E.; Heatherbell, D. A. Influence of processing and storage on the phenolic composition of apple juice. J. Agric. Food Chem. 1990, 38, 1572–1579.
- 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 June 9, 1997. Revised manuscript received October 8, 1997. Accepted October 9, 1997. The study has been carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD program CT95-0158 "Improving the quality and nutritional value of processed foods by optimal use of food antioxidants". It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

### JF9704890

<sup>&</sup>lt;sup>®</sup> Abstract published in Advance ACS Abstracts, December 15, 1997.