

Antioxidant Activity of Berry and Fruit Wines and Liquors

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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 μ M GAE) were slightly superior to reference red grape wines (330–375 μ M GAE) and equally as active as the control antioxidant, α -tocopherol (50 μ 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 peroxy 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 α -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

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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-Amsa et al., 1996) and in a β -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 (C₁₈ 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 H₂O. Prepared wine (0.5 mL) was transferred to the SPE tube, and the tube was washed with 2 mL of H₂O (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 α -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 α -tocopherol (Huang et al., 1996). Reconstituted methanolic berry and fruit wine and liquor extracts (50 and 200 μ 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 2,2,4-trimethylpentane (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 μ M α -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 μ 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 μ M GAE) ranged from 93 to 97%. Wines made of mixtures of black currants and crowberries or bilberries (240–275 μ M GAE) were superior to other berry and fruit wines as well as to the reference red wines and equally as active as α -tocopherol (50 μ 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, cloudberry, mixtures of cloudberry 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 μ 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)^a of Methyl Linoleate Oxidation

| raw materials of berry and fruit wines and liquors | sugar, g/L | alcohol, vol % | total phenolics, mg of GAE/L (after SPE) ^b | μM GAE in 50 μL ^c | % In, 50 μL (PV 400) | % In, 200 μL (PV 400) | % In, 50 μL , (PV 800) | % In, 200 μL (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 |
| 9 black and red currant | 15 | 13 | 1270 (1020) | 300 | 55 | 88 | 33 | 58 |
| 10 black currant | <9 | 12 | 520 (410) | 120 | 93 | 98 | 65 | 98 |
| 11 black currant | <9 | 12 | 1820 (830) | 245 | 93 | 96 | 64 | 96 |
| 12 black currant | 28 | 12 | 995 (800) | 235 | 69 | 91 | 28 | 51 |
| 13 black currant | <45 | 5.5 | 870 (700) | 205 | 37 | 66 | 19 | 13 |
| 14 black currant | 100 | 12 | 1000 (750) | 880 | 8 | 95 | 5 | 91 |
| 15 black and red currant and strawberry (1/1/1) | 20 | 12 | 720 (575) | 170 | 70 | 93 | 41 | 70 |
| 16 black currant and strawberry | 35 | 12 | 695 (550) | 160 | 93 | 95 | 65 | 90 |
| 17 black currant and strawberry | <9 | 12 | 655 (525) | 155 | 60 | 46 | 28 | 19 |
| 18 black currant and strawberry (4/1) | 43 | 13 | 950 (760) | 225 | 95 | 96 | 8 | 97 |
| 19 red currant | 30–50 | 12 | 495 (340) | 1000 | 80 | 96 | 41 | 96 |
| 20 red currant | 100 | 16 | 440 (340) | 100 | 68 | 80 | 39 | 44 |
| 21 apple | >45 | 13 | 470 (375) | 110 | 93 | 94 | 84 | 90 |
| 22 apple | <45 | 12 | 160 (130) | 38 | 59 | 81 | 40 | 42 |
| 23 apple | <45 | 12 | 240 (190) | 56 | 70 | 90 | 36 | 61 |
| 24 apple | <45 | 12 | 260 (210) | 62 | 59 | 86 | 22 | 53 |
| 25 rowanberry and apple | <20 | 13 | 690 (550) | 160 | 76 | 48 | 90 | 93 |
| 26 cowberry and birch sap | 110 | 10 | 775 (600) | 175 | 88 | 96 | 69 | 96 |
| 27 cranberry | <45 | 12 | 680 (540) | 160 | 75 | 20 | 92 | 52 |
| 28 green and white currant | 120 | 11 | 250 (200) | 59 | 83 | 90 | 59 | 65 |
| 29 green and white currant (1/4) | <45 | 12 | 270 (220) | 65 | 53 | 90 | 25 | 64 |
| 30 green and white currants | <20 | 12 | 265 (210) | 62 | 34 | 81 | 14 | 51 |
| 31 strawberry and black currant | 40 | 13.5 | 335 (250) | 73 | 51 | 79 | 41 | 65 |
| 32 rhubarb | 40 | 12 | 125 (100) | 29 | 81 | 65 | 3 | 8 |
| 33 honey | <9 | 4.5 | 91 (73) | 21 | 0 | -10 | -3 | 0 |
| liquors | | | | | | | | |
| 34 cranberry | 300 | 21 | 500 (400) | 120 | 15 | 45 | 3 | 10 |
| 35 cherry | 180 | 21 | 1080 (865) | 255 | 10 | 80 | 30 | 53 |
| 36 arctic bramble | 140 | 15 | 555 (445) | 130 | 24 | 81 | 78 | 78 |
| 37 arctic bramble | 480 | 27.5 | 610 (490) | 145 | 48 | 63 | 26 | 36 |
| 38 strawberry | 130 | 15 | 525 (420) | 125 | 35 | 53 | 21 | 26 |
| 39 strawberry | 320 | 21 | 410 (350) | 105 | -6 | 19 | 3 | 10 |
| 40 rowanberry | >200 | 21 | 545 (435) | 130 | 26 | 93 | 7 | 62 |
| 41 cloudberry | 250 | 21 | 500 (400) | 120 | -30 | 20 | 3 | 10 |
| 42 cloudberry | 335 | 21 | 450 (360) | 105 | -13 | -55 | -5 | -33 |
| 43 cloudberry and red raspberry | 300 | 21 | 415 (330) | 97 | -5 | 15 | 0 | 13 |
| 44 red raspberry and black currant | >200 | 21 | 1050 (840) | 245 | 49 | 79 | 12 | -2 |
| reference grape wines | | | | | | | | |
| 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 3.5 |
| 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 μM α -tocopherol | | | | | 94.6 \pm 4.6 | | | |

^a 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 μM ($\mu\text{mmol/kg}$ of MeLo) GAE when 50 μL of wine was added to MeLo.

μL 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 μM GAE (50 μL of wine added to MeLo) and from 86 to 1200 μM GAE (200 μL) (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 μL of wine extract at control level of 400 mmol of hydroperoxides/kg of MeLo and of 50 and 200

μL 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

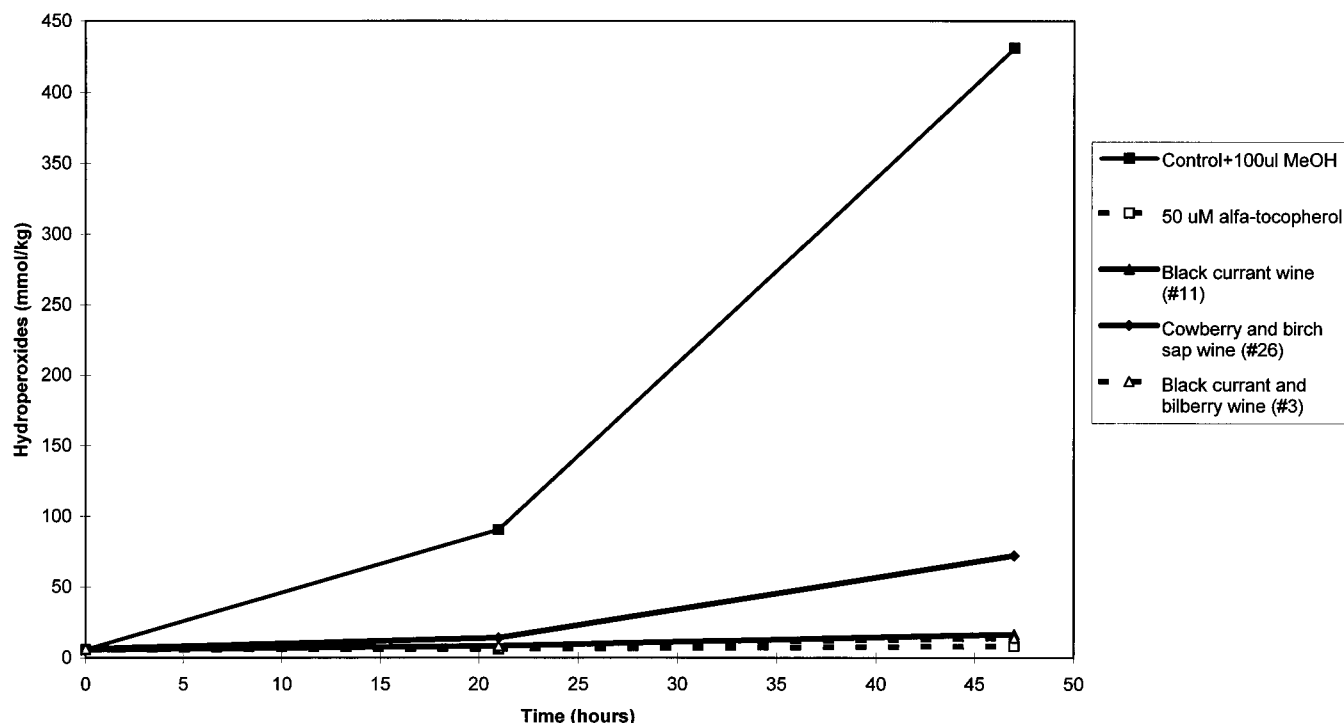


Figure 1. Antioxidant activity of black currant (no. 11), black currant and bilberry (no. 3), and cowberry and birch sap wines (no. 26) (50 μ L) on oxidation of MeLo at 40 $^{\circ}$ C. α -Tocopherol (50 μ M) was used as a control.

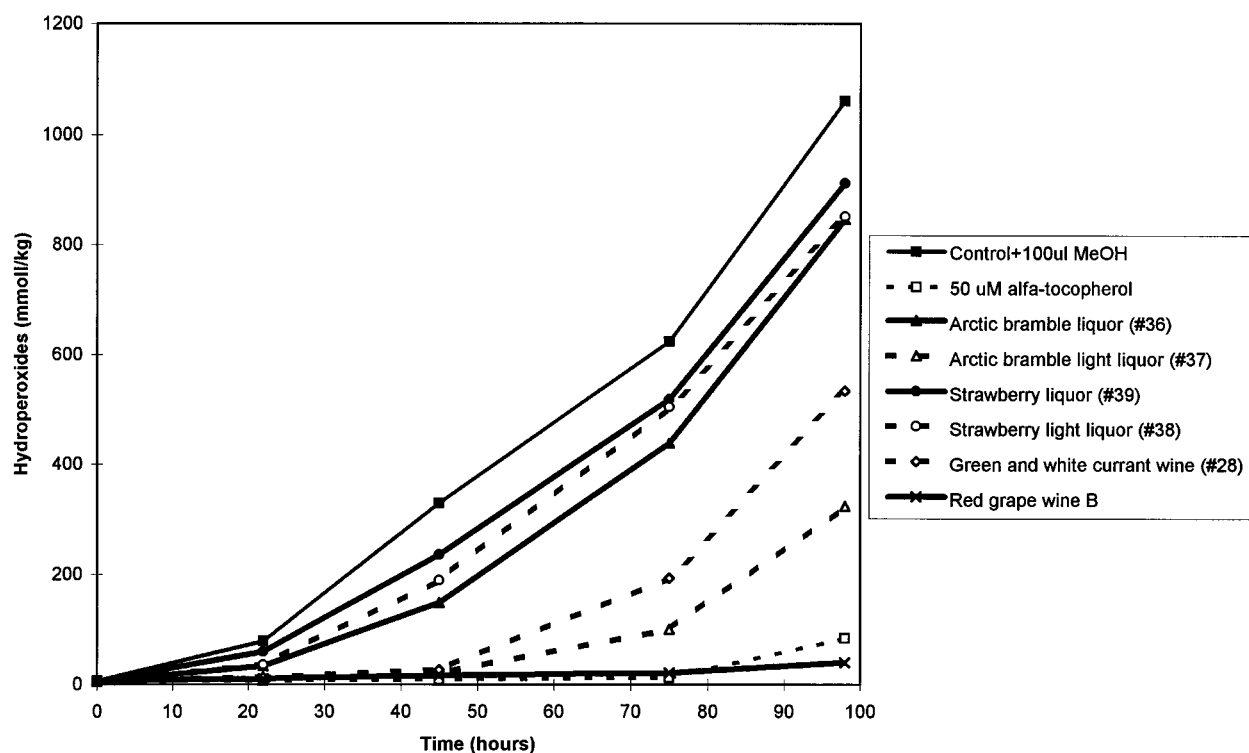


Figure 2. Antioxidant activity of arctic bramble liquor (no. 36) and light liquor (no. 37), strawberry liquor (no. 39) and light liquor (no. 38), and green and white currant wine (no. 28) (50 μ L) on oxidation of MeLo at 40 $^{\circ}$ C. α -Tocopherol (50 μ M) and red wine B (50 μ L) were used as controls.

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 exceptionally 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

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 constituents. 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.

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