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Rubus Fruit Juices Affect Lipid Peroxidation in a *Drosophila melanogaster* Model in Vivo

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The antioxidant capacity of red cloudberry (*Rubus chamaemorus*) juice correlates well with its phenolic content. The red berries have a markedly higher content of anthocyanins, particularly cyanidin and pelargonidin derivatives, than that found in the more common yellow fruit. Conversely, the yellow juice has higher ellagitannin content. A feeding study was conducted to show the in vivo effects of the juices on lipid peroxidation in a sensitive *Drosophila melanogaster* stock. In young female flies there were significant ($P < 0.01$) effects of cloudberry juice on lipid peroxidation. In young male flies significant ($P < 0.05$) effects were found on primary products (hydroxyperoxides) with yellow juice and on secondary products (ketodienes) with red juice. With the red juice, a significant ($P < 0.05$) decrease in ketodienes was found in both young and old males. This study demonstrates that the effects of berry antioxidants on lipid peroxidation are easily and rapidly tested in vivo with the sensitive *Drosophila* model.

KEYWORDS: ***Drosophila*; *Rubus chamaemorus*; cloudberry; lipid peroxidation; anthocyanins; antioxidant**

INTRODUCTION

Phytochemicals of fruits and vegetables in the human diet are suggested to protect against many degenerative conditions, such as coronary disease, stroke, rheumatoid arthritis, and cancer (*1*). However, the wide diversity of bioactive compounds in the human diet has made the research on their effects in vivo difficult. The bioactive compounds most extensively studied are flavonoids and phenolic acids. Anthocyanins are a group of flavonoids responsible for the blue, purple, and red color of fruits. Their phenolic nature provides them with the ability to act as free radical scavengers or antioxidants. Extracts of blackberry, raspberry, strawberry, and other small fruits have been shown to exhibit antioxidant activity in vitro (*2, 3*). Studies on purified compounds have not shown effects similar to those with fruit extracts, possibly due to the poor stability and reactions or interactions with other compounds that may attenuate the effects of a particular compound. The synergistic effect of phenolic compounds has been shown by Rossetto and co-workers, who demonstrated that catechin regenerates the highly efficient antioxidant malvidin 3-glucoside, thereby strongly increasing the antioxidant efficiency of this compound (*4*).

There is a paucity of information regarding the antioxidant properties of anthocyanin-rich materials in vivo. Several studies are available on the absorption and metabolism of anthocyanins (*5*−*10*). However, many open questions remain regarding the bioavailability of fruit antioxidants. Are sufficient amounts of compounds absorbed in a form that affects cellular oxidative damage to the extent that it can be observed in vivo? According to Prior (*11*), even though anthocyanins are detected in human plasma following dietary administration of berry extracts, relatively high dietary levels of anthocyanins are required to observe antioxidant effects in vivo. A rat model with enhanced susceptibility to oxidative damage has been successfully used to assess the ability of anthocyanin-rich extracts from Korean fir cones to modify the lipid peroxidation index (*12*).

The fruit fly, *Drosophila melanogaster*, has been shown to be a valid model system for studying aging and oxidative-stress-related physiological and biochemical phenomena (*13*). Our own past experiences on oxidative aging studies employing *D. melanogaster* (*14*−*16*) led us to anticipate that *Drosophila* might be a fast and simple in vivo model also for establishing and comparing the antioxidative effects of anthocyanin-rich plant materials. We therefore decided to conduct a study by using a preselected *Drosophila* stock, which shows a particularly high level of lipid peroxidation and a shortened lifespan (*17*).

For the study we wanted to select a pair of fruit juices, which would ideally be derived from the same plant species but have clear differences in their anthocyanin profiles and antioxidant

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capacity. The fruit of the wild species *Rubus chamaemorus* (cloudberry) (*18*, *19*) is commonly consumed in northern Europe. The phenolic content and profiles of cloudberry have been studied (*20*, *21*), and the leaf and fruit extracts have been shown to exhibit wide-ranging antimicrobial activities (*22*, *23*). While the normal cloudberry fruit is pearl to yellow in color, a red variant was recently located in eastern Finland. The red fruit was shown to contain much higher levels of anthocyanins than the common cloudberry. This distinct difference provided a means to dissect out and attribute biological activity to anthocyanins by a feeding trial with *Drosophila*.

MATERIALS AND METHODS

Fruit Extracts. Cloudberries (*R. chamaemorus*) were harvested from nature, growing in semiopen mire: the normal yellow berries from Malahvinvaara, Finland, and the red berries from Wärtsilä, Finland. The berries were frozen at −20 °C. While still partly frozen, the berries were crushed with a spoon, and the juices were immediately separated from the seeds and other insoluble material by centrifugation (3000*g*, 10 min, 4 °C). The juices were frozen at −20 °C until they were used for chemical analyses and the *Drosophila* study.

Animals and Diet. As the experimental animal, *D. melanogaster* LA$^-$ stock, preselected during 450 generations for low male mating activity, was used. The stock is maintained at the Department of Genetics, St.-Petersburg State University (St.-Petersburg, Russia). The selection has led to a complex pattern of abnormalities, including a high index of unsaturated fatty acids, high level of lipid peroxidation, and shortened lifespan (*17*). Three-day-old virgin flies were mated (three pairs in each cross) in tubes containing solid nutrient medium composed of 100 g of live yeast, 30 g of sucrose, 30 g of raisins, 30 g of semolina, and 10 g of agar in 1 L of water. After 1 day of mating, the parents were removed from the tubes. The juices were applied on the fourth day of larval development, at which point most of the larvae were at the second developmental stage (second instar). This stage in *Drosophila* development is the most sensitive one to the antioxidative action of different chemicals (*24*). Each tube contained 30−40 larvae. A 200 μL sample of berry juices was applied on top of the agar in tubes containing 8.5 g of medium. The juice was evenly distributed into the agar, giving an approximate content of 2.3% juice. Control animals were given distilled water instead of juice. A larva weighing ∼1.5 mg eats approximately 5 times its own weight, i.e., 7.5 mg, while kept on the culture medium. This amount of the medium thus contained ca. 0.16 mg of juice. One day after hatching from the pupal case, i.e., after a 6-day exposure to the juices (applied on the fourth day), the flies were placed on a fresh medium without the juice. Young and old flies were harvested at the ages of 10 and 21 days, i.e., on the 16th and 27th days after the application of juices to the larvae, respectively.

Lipid Peroxidation. Lipid peroxidation was assessed at the level of primary (conjugated hydroperoxides) and secondary (ketodienes) products from the young and old flies. The fly samples (30 males or 20 females) were weighed. One male and one female weigh approximately 0.9 and 1.5 mg, respectively. The flies were anesthetized with ether and homogenized immediately with glass homogenizer in 1 mL of heptane−2-propanol (1:1, v/v) containing 0.1% butylated hydroxytoluene as antioxidant. The intensity of lipid peroxidation was measured at $\lambda = 232$ nm (hydroperoxides) and $\lambda = 270$ nm (ketodienes) with a Beckman DU-65 spectrophotometer (*25*). The spectrophotometer readings were divided by the fresh weight of the fly samples. There were three replicate samples per study point. The data were compared with ANOVA data after log transformation of the initial figures due to heterogeneity of sample variances.

Antioxidant Capacity in Vitro. The antioxidant capacity in vitro was analyzed from the fruit juices using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (*26*) and by employing the ferric reducing ability of a plasma (FRAP) assay as described by Deighton et al. (*27*). Assays were carried out in triplicate and the mean values calculated.

Total Phenols, Anthocyanins, Ellagitannins, and Vitamin C. Total phenol, anthocyanin, and vitamin C contents of fruit juices were measured as described by Deighton et al. (*27*). Phenolic and anthocyanin contents were expressed as gallic acid and cyanidin 3-glucoside equivalents, respectively, as mg/100 g of juice. Ellagitannin contents were measured as described by Kähkönen et al. (*28*). All assays were carried out in triplicate.

Table 1. Total Phenol, Anthocyanin, Vitamin C, and Ellagitannin Contents and Antioxidant Capacity (As Measured by the FRAP Assay) of Yellow and Red Cloudberry Juices[a]

cloudberry juice	phenols[b]	anthocyanins[b]	vitamin C[b]	ellagitannins[b]	FRAP[c]
red ($t = 0$ days)	230.8 (7.8)	102.6 (2.6)	61.1 (2.4)	251.7 (3.7)	23.6 (1.9)
red ($t = 6$ days)	235.7 (8.2)	100.8 (2.3)	58.7 (2.0)	252.3 (4.2)	22.2 (1.7)
yellow ($t = 0$ days)	92.9 (4.2)	1.2 (0.2)	56.3 (2.2)	391.6 (4.1)	10.5 (0.9)
yellow ($t = 6$ days)	89.9 (3.3)	1.1 (0.2)	55.2 (1.9)	892.1 (4.5)	10.1 (1.1)

[a] All values are the means of triplicate determinations, and the standard errors are in parentheses. Storage at 4 °C. [b] Units of mg/100 g of fresh weight. [c] Units of μmol/g of fresh weight.

Profiling of Phenolic Compounds. The profiling of phenolic compounds from the juices was carried out on a Surveyor LC system (Thermo, U.K.) using a 150 × 4.6 mm i.d. 4 μm Synergi RP-Max C_{18} reversed-phase column (Phenomenex, Macclesfield, U.K.) eluting with a gradient as follows: isocratic elution with 90% solvent A (0.1% formic acid) + 10% solvent B (70% acetonitrile, 0.1% formic acid), 0−1 min; linear gradient to 100% solvent B for 19 min; isocratic elution with 100% B for 3 min; return to 90% A + 10% B by a 2 min linear gradient. The equilibration time between samples was 5 min. The flow rate was 0.25 mL/min, and the injection volume was 10 μL. Primary detection was done by a photodiode array cell scanning over the region 200−700 nm. MS detection was carried out via the LCQ DecaXP ion trap mass spectrometer fitted with an atmospheric pressure chemical ionization (APCI) interface (Thermo, U.K.). The negative ion mode was used since this yielded the greatest number of peaks with good sensitivity. Preliminary analysis was carried out using full-scan, data-dependent MS−MS scanning from *m*/*z* 100 to *m*/*z* 1400. The capillary temperature was 250 °C, the sheath gas and auxiliary gas flow rates were 60 and 10 units/min, respectively, and the source voltage was 2 kV. Unknown compounds were subjected to secondary fragmentation (MS^2). The maximum automatic gain control (AGC) ion storage time was 200 ms, and three microscans were collected per spectrum.

RESULTS AND DISCUSSION

Phenolic Composition of Cloudberry Juices. Clearly, the levels of anthocyanins were very different in the yellow and red cloudberry juices, the levels in the red fruit being almost 85-fold greater compared to the yellow fruit (**Table 1**). Total phenol levels, however, differed only by 2.5-fold, suggesting that anthocyanins were not the dominant phenolic compounds. This is perhaps not surprising as previous reports indicate that up to 80% of the total phenolic content of yellow cloudberry is derived from ellagitannins (*28*).

MS analysis of the anthocyanins (compounds absorbing at 510 nm, **Figure 1**) suggested that, in the red fruit at least, the anthocyanins are cyanidin 3-glucoside (*m*/*z* 448.4; 9.15 min), pelargonidin 3-glucoside (*m*/*z* 432.4; 9.45 min), and pelargonidin 3-sophoroside (*m*/*z* 594.5; 9.45 min). Characteristic fragment ions of the anthocyanin base structures were seen at *m*/*z* 285 (cyanidin) and 269 (pelargonidin), respectively (*29*). Much smaller intensity ions were detected for masses representative of the glycosides. The anthocyanins in the yellow fruit, although present at lower levels, were tentatively identified by LC−MS as cyanidin 3-glucoside. This eluted slightly earlier than with red fruit extracts, possibly due to reduced associations with significant amounts of coeluting anthocyanins.

The nature of the ellagitannins is unclear since their mass spectra are not widely reported, possibly due to their chemical heterogeneity and the fact that they are difficult to purify to homogeneity. However, studies (*29*) on another member of the

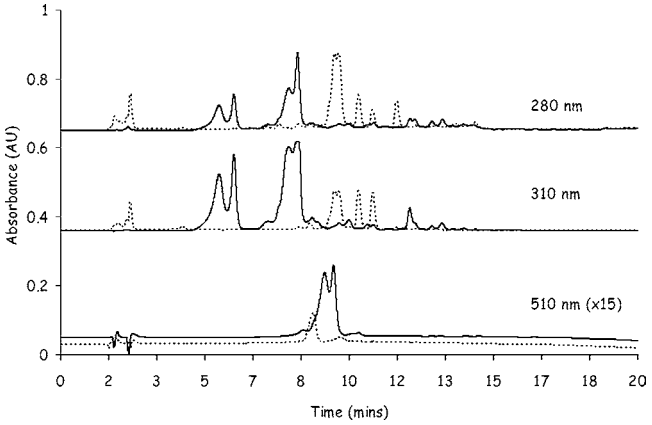

Figure 1. HPLC chromatograms of yellow (---) and red (—) cloudberry juices.

Rubus family, *Rubus idaeus* (red raspberry), clearly identified specific molecular and fragmentation ions associated with both known and, as yet, unknown ellagitannins. These compounds, which to some extent coelute with the anthocyanins (510 nm), are represented by the peaks which elute between 9.2 and 13 min using the chromatographic conditions described (**Figure 1**, λ = 280 and 360 nm). Peaks eluting between 12.8 and 13 min have the reported $(M - H)^-$ fragmentation ions of *m*/*z* 771 and the associated MS^2 fragments of *m*/*z* 591, 505, and 301; the latter are consistent with a base ion of ellagic acid. Interestingly, the known and characterized ellagitannins, sanguiin H-6 and H-10 and lambertianin C, all exhibit very common fragmentation (MS^2) ions at *m*/*z* 1265, 933, and 631, and these were detected and associated with the broad multiplet peak eluting between 9.2 and 9.7 min in the yellow fruit only. Additionally, the peaks eluting at 10.32, 10.8, and 11.64 min also exhibited the ellagic acid MS^2 ion of *m*/*z* 301 and the MS^3 fragment *m*/*z* 257 but have molecular ions previously reported to be associated with less commonly seen pentose conjugates: at 11.64 min ellagic acid−pentose conjugate (*m*/*z* − 433) and ellagic acid acetyl−pentoside (*m*/*z* − 475) (*29*).

The remaining predominant peaks in the chromatogram of the red fruit, the peaks at 5.5, 6.0, and 7.5−8.0 min, remain unidentified. They do not display the common ellagitannin peak at *m*/*z* 301; rather the common ions are at *m*/*z* 353.1, 236.8, 191.2, and 179.3. Despite absorption at 310 nm, often characteristic of the presence of substituted cinnamates (*30*), no corresponding ions were found.

The overall differences found between the yellow and red fruit juices were (1) a much higher content of anthocyanins, particularly cyanidin and pelargonidin derivatives, and other unidentified nonanthocyanin phenolic compounds in the red berries, (2) a much higher content of ellagitannins in the yellow berries, (3) an over 2-fold greater level of phenolic compounds in the red berries compared to the yellow berries, and (4) similar contents of vitamin C.

In Vitro Antioxidant Capacities. The juices from both yellow and red cloudberry fruits exhibited significant and distinct antioxidant levels. The ability of the juices to scavenge DPPH radicals was almost equivalent (**Figure 2**) and followed a standard dilution response. Regression analysis showed that the juices had to be diluted 12.5-fold to lose 50% of their antioxidant capacities. No significant differences between regression coefficients for yellow and red juices were detected. In contrast, there was a 2.2-fold difference between red and yellow juice antioxidant capacity as analyzed using the FRAP assay (**Table 1**), which measures the ability of the juice antioxidants to donate

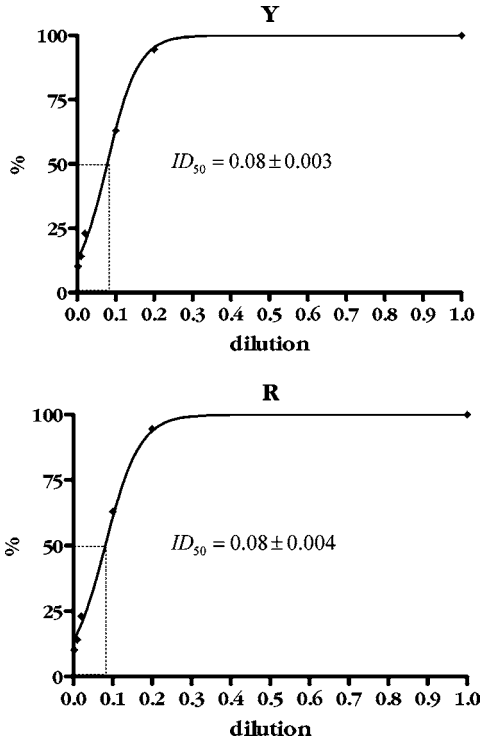

Figure 2. Fitted regression curves of in vitro antioxidant activity of the yellow (Y) and red (R) cloudberry juices: percentage of suppression of DPPH activity vs dilution of juices.

electrons. The results suggest that the two antioxidant assays do not measure the same moieties in the juices. The difference in the antioxidant capacity analyzed with the FRAP assay correlated well with the difference found in total phenolic compounds in the juices. This is in agreement with published data (*27*, *31*).

Lipid Peroxidation in *Drosophila*. Cloudberry juices were given to *Drosophila* during the second and third larval stages. Lipid peroxidation was measured from the 10-day- and 21-day-old adult winged flies. In the 10-day-old (young) female flies there were significant ($P < 0.01$) effects of both red and yellow cloudberry juice on primary (hydroperoxides) as well as on secondary (ketodienes) products of lipid peroxidation (**Figure 3**). In the 10-day-old male flies significant ($P < 0.05$) effects were found on primary products with yellow juice only and on secondary products with red juice only. This suggests that the different phenolic compounds may affect the two stages of lipid peroxidation differently. In the 21-day-old flies, no significant effects on the primary products of lipid peroxidation were observed. Interestingly, contrasting effects on secondary products were found. The level of ketodienes was higher in flies (both sexes) that had received yellow juice compared to the controls, the effect being significant ($P < 0.05$) in the female group. With the red juice, a statistically significant ($P < 0.05$) decrease in ketodienes was found in the male group. This was the only clear indication of the antioxidative effect of the juices in the 21-day-old fly groups.

To evaluate the sources of variability, the data were subjected to two-way ANOVA (**Tables 2** and **3**). The cloudberry juice type had a significant ($P < 0.01$) effect on the secondary products but not primary products of lipid peroxidation. The levels of both primary ($P = 0.010$) and secondary ($P < 0.001$) lipid peroxidation products were affected by significant juice−age interaction.

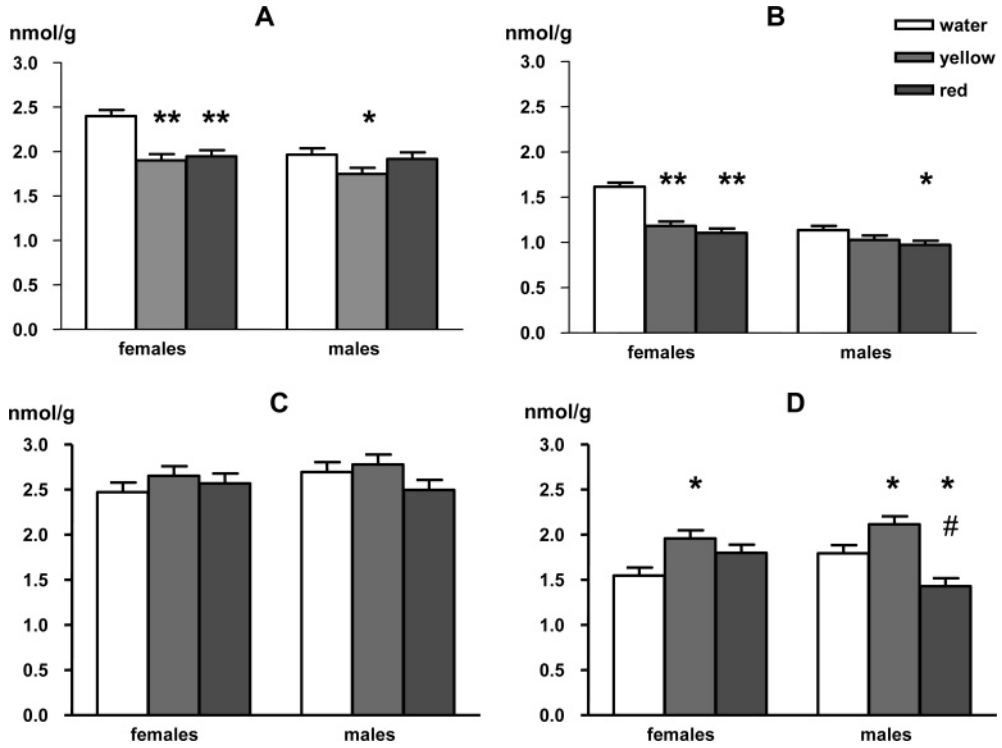

Figure 3. Lipid peroxidation in 10-day-old (**A**, **B**) and 21-day-old (**C**, **D**) female and male *Drosophila* flies after the larvae were provided with red or yellow cloudberry juice. Hydroperoxides are shown on the left (**A**, **C**) and ketodienes on the right (**B**, **D**). Key: *, $P < 0.05$ compared to water; **, $P < 0.01$ compared to water; #, $P < 0.05$ compared to the other juice; calculated by the Bonferroni post hoc test. The values are means of triplicate determinations ± SEM.

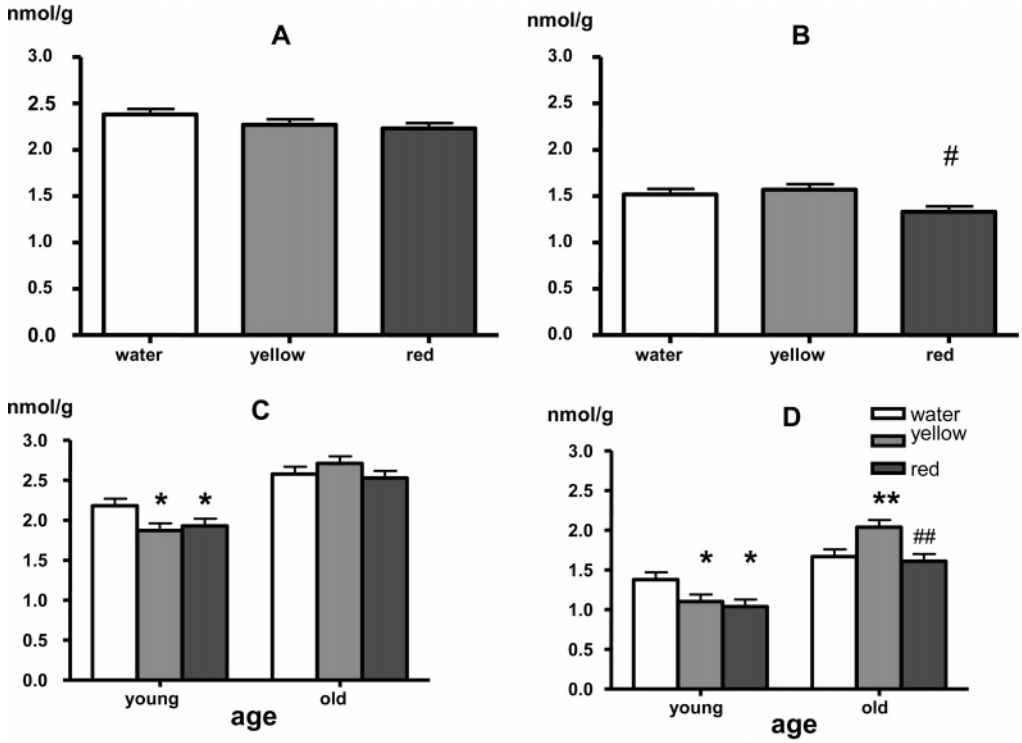

Figure 4. Mean plots for juice types (**A**, **B**) and juice–age interactions (**C**, **D**), derived from the analysis of variance. Hydroperoxides are shown on the left (**A**, **C**) and ketodienes on the right (**B**, **D**). Key: *, $P < 0.05$ compared to water; **, $P < 0.01$ compared to water; #, $P < 0.05$ compared to the other juice; ##, $P < 0.01$ compared to the other juice; calculated by the Bonferroni post hoc test. The values are means of triplicate determinations ± SEM.

In general, the red cloudberry juice appeared to be more effective in decreasing the level of secondary lipid peroxidation products. There were no overall differences in the effects of the juices on the hydroperoxide level (**Figure 4A**), but the level of ketodienes was significantly ($P < 0.05$) lower after the flies were fed red juice as compared to the yellow juice (**Figure 4B**). The juice−age interactions seemed similar for young flies, but in old flies the level of ketodienes was decreased by red juice as compared with the yellow juice (**Figure 4C,D**). Thus, the antioxidative effect appears to last longer after withdrawal of

Table 2. Analysis of Variance for Conjugated Hydroperoxides (log)

source of variation	SS_t	Df	MS_b	*F* ratio	*P* value
main effects					
A, juice type	0.0313	2	0.0156	2.12	NS
B, sex	0.0098	1	0.0098	1.33	NS
C, age	0.6963	1	0.6963	94.29	<0.001
interactions					
AB, juice–sex	0.0030	2	0.0015	0.21	NS
AC, juice–age	0.0804	2	0.0402	5.45	0.010
BC, sex–age	0.0382	1	0.0382	5.18	0.031
residual	0.1920	26	0.0073		
total	1.0512	35			

Table 3. Analysis of Variance for Ketodienes (log)

source of variation	SS_t	Df	MS_b	*F* ratio	*P* value
main effects					
A, juice type	0.1853	2	0.0926	8.95	<0.01
B, sex	0.1060	1	0.1060	10.24	<0.01
C, age	1.5472	1	1.5471	149.45	<0.001
interactions					
AB, juice–sex	0.0341	2	0.0171	1.65	NS
AC, juice–age	0.2516	2	0.1258	12.15	<0.001
BC, sex–age	0.0872	1	0.0872	8.42	<0.01
residual	0.3848	26	0.0104		
total	2.5962	35			

the red juice than after withdrawal of the yellow juice, the effect being more prominent on the secondary products of lipid peroxidation. These findings are in agreement with other *D. melanogaster* studies, which aimed at establishing relationships between dietary components and oxidative stress and aging. Bonilla et al. (*32*) found that supplementation of the *D. melanogaster* diet with melatonin, a hormone with antioxidant and free radical scavenging activity, increased the maximum lifespan by 33% and was accompanied by an increase in resistance to exposure to paraquat, a compound whose metabolism in vivo generates superoxide (*33*). Similarly, other studies (*34*) have shown that supplementation of the *D. melanogaster* diet with phenolic compounds such as propyl gallate and nitrophenol produced significant increases in the lifespan, namely, 34.4% and 12.3%, respectively. Interestingly, vitamin C only produced an increase of 1.7%. In the present study only a minor difference in the vitamin C contents of the two juices was found.

Clearly, the effect presented here, i.e., retardation of lipid peroxidation due to cloudberry phenolics (specifically anthocyanins), adds weight to the increasing body of evidence supporting the need to increase the amount of anthocyanin-rich fruits in the diet. We now aim to dissect out the effects attributable to the specific chemical classes present in the yellow and red cloudberry and to purify and characterize the unknown, 310 nm absorbing component present in the red fruit. Further *D. melanogaster* feeding studies should allow at least preliminary steps to be taken toward relating food compositional chemistry to beneficial effects on aging and oxidative metabolism.

LITERATURE CITED

(1) Anon. *Diet, nutrition and the prevention of chronic diseases*; Report of a Joint WHO/FAO Expert Consultation, WHO Technical Report Series, No. 916; WHO: Geneva, Austria, 2003; p 160.

(2) Moyer, R. A.; Hummer, K. E.; Finn, C. E.; Frei, B.; Wrolstad, R. E. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. *J. Agric. Food Chem.* **2002**, *50*, 519−525.

(3) Wang, S. Y.; Lin, H. S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140−146.

(4) Rossetto, M.; Vanzani, P.; Mattivi, F.; Lunelli, M.; Scarpa, M.; Rigo, A. Synergistic antioxidant effect of catechin and malvidin 3-glucoside on free radical-initiated peroxidation of linoleic acid in micelles. *Arch. Biochem. Biophys.* **2002**, *408*, 239−245.

(5) Ichianagi, T.; Mamunur Rahman, M.; Kashiwada, Y.; Ikeshiro, Y.; Shida, Y.; Hatano, Y.; Matsumoto, H.; Hirayama, M.; Tsuda, T.; Konishi, T. Absorption and metabolism of delphinidin 3-*O*-*β*-d-glucopyranoside in rats. *Free Radical Biol. Med.* **2004**, *36*, 930−937.

(6) Cao, G.; Muccitelli, H. U.; Sanchez-Moreno, C.; Prior, R. L. Anthocyanins are absorbed in glycated forms in elderly women: a pharmacokinetic study. *Am. J. Clin. Nutr.* **2001**, *73*, 920−926.

(7) Wu, X.; Cao, G.; Prior, R. L. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *J. Nutr.* **2002**, *132*, 1865−1871.

(8) Felgines, C.; Texier, O.; Besson, C.; Fraisse, D.; Lamaison, J. L.; Remesy, C. Blackberry anthocyanins are slightly bioavailable in rats. *J. Nutr.* **2002**, *132*, 1249−1253.

(9) Miyazawa, T.; Nakagawa, K.; Kudo, M.; Muraishi, K.; Somcya, K. Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. *J. Agric. Food Chem.* **1999**, *47*, 1083−1091.

(10) Matsumoto, H.; Inaba, H.; Kishi, M.; Tominaga, S.; Hirayama, M.; Tsuda, T. Orally administered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in the blood as the intact forms. *J. Agric. Food Chem.* **2001**, *49*, 1546−1551.

(11) Prior, R. L. Fruits and vegetables in the prevention of cellular oxidative damage. *Am. J. Clin. Nutr.* **2003**, *78*, 570−578.

(12) Ramirez-Tortosa, C.; Andersen, O. M.; Gardner, P. T.; Morrice, P. C.; Wood, S. G.; Duthie, S. J.; Collins, A. R.; Duthie, G. G. Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. *Free Radical Biol. Med.* **2001**, *31*, 1033−1037.

(13) Schmookler-Reis, R. J.; Ebert, R. H. Genetics of ageing: Current animal models. *Exp. Gerontol.* **1996**, *31*, 69−81.

(14) Mylnikov, S. V.; Smirnova, A. N.; Oparina, T. I.; Bliudzin, I. A.; Kaidanov, L. Z. The intensity of the peroxidation of lipids and their fatty acid composition in Drosophila melanogaster strains differing in their adaptive value. *Zh. Evol. Biokhim. Fiziol.* **1997**, *33*, 12−16.

(15) Anisimov, V. N.; Mylnikov, S. V.; Oparina, T. I.; Khavinson, V. K. Effect of melatonin and pineal peptide preparation epithalamin on life span and free radical oxidation in Drosophila melanogaster. *Mech. Ageing Dev.* **1997**, *97*, 81−91.

(16) Anisimov, V. N.; Mylnikov, S. V.; Khavinson, V. K. Pineal peptide preparation epithalamin increases the lifespan of fruit flies, mice and rats. *Mech. Ageing Dev.* **1998**, *103*, 123−132.

(17) Kaidanov, L. Z.; Mylnikov, S. V.; Iovleva, O. V.; Galkin, A. P. Directed character of genetic variation during long-term selection of *Drosophila melanogaster* strains for adaptively important characters. *Genetika* **1994**, *30*, 1085−1096.

(18) Saastamoinen, O.; Kangas, K.; Aho, H. The picking of wild berries in Finland in 1997 and 1998. *Scand. J. For. Res.* **2000**, *15*, 645−650.

(19) Martinussen, I.; Svenson, L.; Junttila, O.; Rapp, K. *In vitro* propagation of cloudberry (*Rubus chamaemorus* L.). *Plant Cell, Tissue Organ Cult.* **2004**, *78*, 43−49.

(20) Häkkinen, S.; Kärenlampi, S.; Mykkäne, H.; Heinonen, M.; Törrönen, R. Ellagic acid content in berries: Influence of domestic processing and storage. *Eur. Food Res. Technol.* **2000**, *212*, 75−80.

(21) Häkkinen, S.; Kärenlampi, S.; Heinonen, M.; Mykkänen, H.; Törrönen, R. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *J. Agric. Food Chem.* **1999**, *47*, 2274−2279.

(22) Puupponen-Pimiä, R.; Nohynek, L.; Meier, C.; Kähkönen, M.; Heinonen, M.; Hopia, A.; Oksman-Caldentey, K.-M. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.* **2001**, *90*, 494−507.

(23) Rauha, J. P.; Remes, S.; Heinonen, M.; Hopia, A.; Kähkönen, M.; Kujala, T.; Pihlaja, K.; Vuorela, H.; Vuorela, P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int. J. Food Microbiol.* **2000**, *56*, 3−12.

(24) Emanuel', N. M.; Obukhova, L. K.; Smirnov, L. D.; Bunto, T. V. Experimental increase in lifespan due to the action of 2-ethyl-6-methyl-3-hydroxypyridine chlorhydrate *Dokl. Akad. Nauk SSSR* **1976**, *226*, 961−963.

(25) Stalnaya, I. D. Methods for detecting dien conjugation in fatty acids (in Russian). In *Sovremennye metody biokhimii* (*Modern Methods in Biochemistry*); Orekhovich, V. N., Ed.; Meditsyna: Moscow, Russia, 1977; pp 63−66.

(26) Glavind, J. Antioxidants in animal tissue. *Acta Chem. Scand.* **1963**, *17*, 1635−1640.

(27) Deighton, N.; Brennan, R.; Finn, C.; Davies, H. V. Antioxidant properties of domesticated and wild *Rubus* species. *J. Sci. Food Agric*. **2000**, *80*, 1307−1313.

(28) Kähkönen, M. P.; Hopia, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076−4082.

(29) Mullen. W.; Yokotab, T.; Lean, M. E. J.; Crozier, A. Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC-MS^n. *Phytochemistry* **2003**, *64*, 617−624.

(30) Bunzel, M.; Ralph, J.; Funk, C.; Steinhart, H. Isolation and identification of a ferulic acid dehydrotrimer from saponified maize bran insoluble fiber. *Eur. Food Res. Technol.* **2003**, *217*, 128−133.

(31) Luximon-Ramma, A.; Bahorun, T.; Alan Crozier, A. Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *J. Sci. Food Agric.* **2003**, *83*, 496−502.

(32) Bonilla, E.; Medina-Leendertz, S.; Díaz, S. Extension of life span and stress resistance of *Drosophila melanogaster* by long-term supplementation with melatonin. *Exp. Gerontol.* **2002**, *37*, 629−638.

(33) Fridovich, I.; Hassan, H. M. Paraquat and the exacerbation of oxygen toxicity. *Trends Biochem. Sci.* **1979**, *4*, 113−115.

(34) Le Bourg, E. Oxidative stress, aging and longevity in *Drosophila melanogaster*. *FEBS Lett.* **2001**, *498*, 183−186.

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