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Chemical Compositions, Antioxidant Capacities, and Antiproliferative Activities of Selected Fruit Seed Flours

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Seed flours from black raspberry, red raspberry, blueberry, cranberry, pinot noir grape, and chardonnay grape were examined for their total fat content, fatty acid composition, total phenolic content (TPC), total anthocyanin content (TAC), radical scavenging capacities against the peroxyl (ORAC) and stable DPPH radicals, chelating capacity against Fe^{2+}, and antiproliferative activities using the HT-29 colon cancer cell line. Significant levels of fat were detected in the fruit seed flours and their fatty acid profiles may differ from those of the respective seed oils. Cranberry seed flour had the highest level of α-linolenic acid (30.9 g/100 g fat) and the lowest ratio of n-6/n-3 fatty acids (1.2/1). The ORAC value of the chardonnay seed flour was 1076.4 Trolox equivalents μmol/g flour, and its TPC was 186.3 mg gallic acid equivalents/g flour. These values were 3−12 times higher than the other tested fruit seed flours. Furthermore, the ORAC value was significantly correlated to the TPC under the experimental conditions ($P < 0.05$). These fruit seed flours also differed in their TAC values and Fe^{2+}-chelating capacities. In addition, black raspberry, cranberry, and chardonnay grape seed flour extracts were evaluated for their antiproliferative effects using HT-29 colon cancer cells. All three tested seed flour extracts significant inhibited HT-29 cell proliferation. The data from this study suggest the potential of developing the value-added use of these fruit seed flours as dietary sources of natural antioxidants and antiproliferative agents for optimal human health.

KEYWORDS: Antioxidant; fatty acid; radical scavenging activity; ORAC; fruit seed; HT-29 cancer cell line.

INTRODUCTION

Growing scientific evidence suggests that a number of food components may reduce the risk of chronic diseases and improve general human health (*1*−*5*). These components include, but are not limited to vitamins, carotenoids, ω-3 fatty acids, dietary fibers, and natural antioxidants such as anthocyanins and other polyphenolics (*4*−*9*). Dietary natural antioxidants are believed to play a crucial role in human health by preventing life important biological molecules such as DNA and membrane lipids from oxidative damage (*10*, *11*). Novel food ingredients rich in natural antioxidants and other beneficial factors are in high demand for improving food quality and optimizing human health.

Fruit seeds are byproducts from fruit processing, and seed flour is the primary byproduct from seed oil production. Our recent study showed that black raspberry seed flour obtained from the cold-pressing procedure may contain a significant level of antioxidants (*8*). The black raspberry seed flour was extracted with 50% acetone at ambient temperature and with 100% ethanol using a Soxhlet extractor. Both 50% acetone and 100% ethanol extracts were able to directly react with and quench DPPH and ABTS•$^+$ radicals and contained significant levels of phenolic compounds (*8*). In addition, the black raspberry flour contained 5.3% oil on a per weight basis, and α-linolenic acid [18:3(n-3)] comprised approximately 33% of the total fatty acids in the oil (*8*). α-Linolenic acid is the precursor for biosynthesis of longer chain n-3 polyunsaturated fatty acids, eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)]. EPA and DHA may reduce the risk of cancer, heart disease, hypertension, and autoimmune disorders (*12*−*17*). These data suggest the potential of developing novel uses of fruit seed flours as food ingredients rich in beneficial food factors for improving human diets, while enhancing the profitability of fruit production and processing industries. Additional research is required to investigate fruit seed flours for their contents of health beneficial factors to promote their value-added utilization as beneficial food ingredients.

The present study was conducted to investigate the seed flours of black raspberry (*Rubus occidentalis* L., cv Jewel), red

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raspberry (*Rubus ideaus*), blueberry (*Vaccinium corymbosum*), pinot noir grape (*Vitis vinifera*), chardonnay grape (*Vitis vinifera*), and cranberry (*Vaccinium macrocarpon*) for their total phenolic and total anthocyanin contents, total fat and fatty acid composition, free radical scavenging capacities, chelating activity, and antiproliferative activity against human colon cancer cells. The data obtained from this study will be used to promote the potential utilization of these edible seed flours in food products for improving human health.

MATERIALS AND METHODS

Materials. Flours from black raspberry, red raspberry, blueberry, cranberry, pinot noir grape, and chardonnay grape seeds were provided by the Badger Oil Co. (Spooner, WI). These fruit seed flours were the solid residues from the cold-pressing seed oil production. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), ethylenediaminetetraacetate (EDTA), potassium chloride, sodium acetate, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO); β-cyclodextrin (RMCD) was purchased from Cyclolab R & D Ltd. (Budapest, Hungary), and 2,2′-azobis (2-aminopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). For cell culture, disposable culture ware was purchased from Corning Glass Works (Corning, NY). Cell culture media [McCoy's 5A medium modified with L-glutamine, antibiotic/antimycotic, and fetal bovine serum (FBS)] and 0.25% trypsin with 0.9 mM EDTA were purchased from Invitrogen (Carlsbad, CA). HT-29 human colon cancer cells were purchased from American Type Culture Collection (Rockville, MD). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Extraction and Testing Sample Preparation. One gram of seed flour was extracted with 10 mL of 50% acetone at ambient temperature. The extracts were examined for total phenolic content (TPC), chelating activity, oxygen radical absorbance capacity (ORAC), DPPH•-scavenging activity, and cancer cell growth inhibition (*8*). For estimating the total anthocyanin concentration (TAC), each flour sample was extracted with acidic methanol containing 2% 12 M HCl (v/v). Solvents, in a known volume of each extract, were removed by evaporation. The solid residue was quantitatively redissolved in DMSO to obtain a final concentration of 1 g of flour equivalents per mL of DMSO. All extracts were kept in the dark under nitrogen until analysis.

Total Phenolic Contents. Folicin−Ciocalteu (FC) reagent was used to determine the TPC of the fruit seed flour extracts following a laboratory procedure previously described by Yu and others (*18*). Briefly, the reaction mixture contained 250 μL of fresh FC reagent, 750 μL of 20% Na_2CO_3, 50 μL of the fruit seed flour extract or standard, and 3 mL of pure H_2O. Absorbance was determined at 765 nm after 2 h of reaction at ambient temperature and used to calculate the phenolic contents in the seed flours, with gallic acid as the standard. The FC reagent was freshly prepared by refluxing a mixture of sodium molybdate, sodium tungstate, 85% phosphoric acid, and concentrated HCl for 10 h. This was followed by reaction with lithium sulfate and oxidation with a few drops of bromine. The resulting solution was then filtered and ready for assay.

Total Anthocyanin Content. The TAC was determined by a pH differential method (*19*−*21*). Anthocyanins undergo a reversible structural transformation from pH 1 to pH 4.5, and consequently, they absorb light at 510 nm at pH 1 but negligibly absorb at pH 4.5. Degraded polymeric anthocyanins absorb light at both pH 1 and pH 4.5; therefore, they are not measured in this experiment. Absorbance at 700 nm was measured to correct for haze. Extracts were added to two buffer systems at the same dilution. The first buffer system was 0.025 M potassium chloride at pH 1.0, and the second buffer system was 0.4 M sodium acetate at pH 4.5. After adding the sample extract, buffer systems were equilibrated for 15 min and then absorptions were read at 510 and 700 nm. The calculated absorption was determined by the equation: $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$ and the TAC in the testing solution was calculated as cyanidin-3-glucoside equivalents (CGE mg/L): $(A \times 449.2 \times \text{dilution factor} \times 1000)/(\epsilon \times 1)$, with the $\epsilon = 26\,900$. The molecular weight of cyanidin-3-glucoside is 449.2 g/mol.

Fatty Acid Composition. Oils were extracted from the flour samples using a Soxhlet apparatus, and petroleum ether was the solvent. Fatty acid methyl esters (FAME) were prepared from the oils according to the previously described method using HCl in MeOH following saponification (*22*). Fatty acid compositions were analyzed by a Shimadzu GC-2010 with a FID and a Shimadzu AOC-20i autosampler (Shimadzu, Columbia, MD). A Supelco 2380 column, 30 m × 0.25 mm i.d. with a 0.20 μm film thickness (Supelco Inc., Bellefonte, PA) was used with helium as the carrier gas at a flow rate of 0.8 mL/min. Injection volume was 1 μL at a split ratio of 10/1. Time and temperature ramps began with an initial oven temperature of 142 °C increasing 6 °C/min to 184 °C, held for 3 min, and then increased 6 °C/min to 244 °C (*23*). Identification of the individual fatty acids was conducted by comparing GC retention time with that of the authorized pure individual commercial compounds.

Oxygen Radical Absorbance Capacity. The ORAC was determined using the protocol previously described (*24*, *25*). Fluorescein was used as the fluorescent probe. The assay mixture contained 0.067 μM of fluorescein, 60 mM of AAPH, 300 μL of flour extract or 50% acetone for the reagent blank. The fluorescence of an assay mixture was recorded every minute, and the area under the curve of fluorescence vs time plot was calculated and compared against a standard curve prepared with Trolox. The ORAC value was expressed as Trolox equivalents (TE) in micromole per gram of the fruit seed flour. Triplicate measurements were conducted.

DPPH•-Scavenging Activity. The stable DPPH (2,2-diphenyl-1-picryhydrazyl) radical scavenging capacities of the cold-pressed seed flour extracts were analyzed following a previously described procedure (*26*). A freshly prepared DPPH•−50% acetone solution was mixed with an equal volume of seed flour extract to start the radical−antioxidant reaction. The initial DPPH• concentration was 100 μM and the total reaction volume was 2.0 mL. Absorbance was measured at 517 nm against a blank of 50% acetone and used to estimate the remaining radical levels according to the standard curve. The seed flour extracts were tested for their $ED_{50-DPPH}$ concentrations at 20 min of reaction. The $ED_{50-DPPH}$ is the concentration of extract needed to quench 50% of the DPPH radicals under experimental conditions at a predetermined time. Time and dose dependencies of extracts and DPPH• reactions were demonstrated by plotting the percent of DPPH• remaining against time for each level of the seed flour extract tested.

Chelating Capacity. Chelating capacity against Fe^{2+} was measured using the 2,2-bipyridyl competition assay (*27*). The reaction solution contained 30 μL of 1.8 mM $FeSO_4$, 400 μL of standard or sample solution, 200 μL of pH 7.4 Tris-HCl buffer, 50 μL of 0.1% 2,2′-bipyridal in 0.2 M HCl, and 200 μL of 7% RMCD in 50% acetone. The absorbance was read at 522 nm. EDTA was used as the standard. Measurements were conducted in triplicate.

HT-29 Colon Cancer Cell Proliferation Inhibition. The HT-29 human colorectal adenocarcinoma cell line characterized by Fogh (*28*) was propagated in T-150 flasks in McCoy's 5A media containing 10% FBS and 1% antibiotic/antimycotic. Flasks were incubated at 37 °C in a humidified atmosphere at 5% CO_2 (*29*, *30*).

Cell proliferation was examined following a modified procedure using 12-well plates (*30*). The initial cell number was 2.5, 3.0, and 5.0 × 10^4 per well for the black raspberry, cranberry, and chardonnay grape seed flour extracts, respectively. After 24 h of incubation in the control media at 37 °C in a humidified atmosphere containing 5% CO_2, cells were treated with media containing the DMSO solution of the fruit seed flour extracts at two levels, while the control cells were treated with same volume of DMSO. The two dose levels were 3 and 6 mg flour equivalents/mL culture media for all tested fruit seed flour extracts. Media and treatments were changed daily, and live cells were counted on day 1 through day 4 of treatment.

Statistical Analysis. Data were reported as mean ± standard deviation ($n = 3$). Analysis of variance and least significant difference tests (SPSS for Windows, Version Rel. 10.0.5, 1999, SPSS Inc., Chicago, IL) were conducted to identify differences among means,

Table 1. Phytochemical Compositions of Studied Cold-Pressed Edible Seed Flours[a]

	TPC	TAC	total fat (g/100 g flour)
black raspberry	41.2 ± 1.20 c	61.3 ± 5.85 a	NA
red raspberry 1	25.1 ± 2.26 d	0.0 ± 0.00 e	4.6
red raspberry 2	NA	NA	5.4
blueberry 1	15.8 ± 0.63 e	7.4 ± 0.35 c	3.7
blueberry 2	NA	NA	2.8
cranberry	14.6 ± 0.04 f	13.8 ± 1.39 b	6.8
pinot noir grape	55.5 ± 11.23 b	0.28 ± 0.10 d	1.2
chardonnay grape	186.3 ± 5.13 a	6.85 ± 0.29 c	5.3

[a] Red raspberry1 and 2 and blueberry 1 and 2 are two different samples from the same variety. TPC is the total phenolic content in the respective fruit seed flours and is measured as milligrams of gallic acid equivalents (GAE) per gram of flour. TAC is the total anthocyanin content and is measured as milligrams of cyanidin-3-glucoside equivalents per 100 g of seed flour. Values in columns with different letters are significantly different ($P < 0.05$).

while a Pearson correlation test was conducted to determine the correlations among means. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Total Phenolic Content. The TPC values of the fruit seed flours ranged from 14.5 to 186.3 mg of gallic acid equivalents per gram of flour (mg GAE/g) (**Table 1**). The chardonnay grape seed flour had the highest TPC value among all tested seed flours, and pinot noir grape seed flour had the next highest value at 55.5 mg GAE/g. The TPC values of these two grape seed flours were higher or comparable to that of 29.9−57.1 mg GAE/g seed detected in the 10 muscadine grape varieties (*31*). Black raspberry seed flour had a TPC value of 41.2 mg GAE/g (**Table 1**) and was comparable to that of 45.6 mg GAE/g previously reported for black raspberry seed flour (*8*). Furthermore, the TPC values of red raspberry and black raspberry seed flours were lower than the TPC in the whole dried fruits, respectively (*32*). The red raspberry and black raspberry fruits had TPC values of 36.9 and 57.6 mg GAE/g, and the TPC values of the red raspberry and black raspberry seed flours were 25.1 and 41.2 mg GAE/g, respectively (**Table 1**). These data suggest that these fruit seeds may potentially serve as natural sources for dietary phenolic compounds. In addition, the TPC values of the flours were significantly correlated to their ORAC values ($r = 0.992$, $P < 0.01$), suggesting that the phenolic compounds contribute to their oxygen radical absorbing capacities.

Total Anthocyanin Content. The highest TAC of 61.3 mg of cyanidin 3-glucoside equivalents per 100 g of flour (CGE mg/100 g) was detected in the black raspberry seed flour, and no anthocyanin was detected in the red raspberry flour under the present experimental conditions (**Table 1**). The TAC value of the black raspberry seed flour from this study was lower than that found in the whole dried black raspberry fruit at the level of 3465 CGE mg/100 g (*32*). This TAC value is greater than that detected in fresh whole raspberry fruits examined by Liu and others on a per weight basis (*33*). Liu and others reported that four varieties of whole fresh raspberry had a TAC range of 0.17−57.6 CGE mg/100 g (*33*). This study also found significant levels of TAC in the cranberry, blueberry, and chardonnay grape seed flours at a range of 6.9 to 7.4 CGE mg/100 g (**Table 1**). The TAC value of 13.8 CGE mg/100 g for cranberry seed flour was lower but comparable to that of 19.8−65.6 CGE mg/100 g reported for 10 different cultivars of fresh cranberry (*34*). In this study, blueberry seed flour had a TAC value of 7.4 CGE mg/100 g, which is much lower than that observed in the 87 highbush blueberry cultivars with a range of 890−3310 CGE mg/100 g on a fresh weight basis (*4*). Additionally, the TAC values of grape seed flours from this study were lower than that of 68.5−150.7 CGE mg/100 g reported for four varieties of grapes on a per fresh weight basis (*35*). These data indicate that anthocyanins are not concentrated in fruit seeds, although black raspberry seed may contain a significant level of anthocyanins.

Fatty Acid Composition. Cranberry seed flour had the highest oil content among the samples containing 6.8% oil on a per weight basis (**Table 1**). The cranberry seed flour also had the highest α-linolenic acid [18:3(n-3)] concentration, containing more than 30 g/100 g oil and had a very low ratio of n-6 to n-3 fatty acids at approximately 1.2:1 (**Table 2**). This level is similar to that of 33.2 g/100 g oil detected in the black raspberry seed flour (*8*). The n-6 to n-3 fatty acid ratio was about 1.6:1 for the black raspberry seed flour (*8*). The chardonnay grape seed flour had the highest content of linoleic acid [18:2(n-6)] at approximately 66 g/100 g oil and was followed by that of the cranberry seed flour at 39.9 g/100 g oil. All of the tested seed flours had relatively high concentrations of oleic acid [18:1(n-9)] containing 19.2−46.1 g/100 g fat (**Table 2**). The linoleic acid concentration in the pinot noir grape seed flour was determined to be 13 g/100 g oil. This concentration was notably lower than reported grape seed oils that have demonstrated linoleic acid from 50.1 to 77.8 g/100 g oil (*36*). In this study, the oils from red raspberry and blueberry seed flours contained total saturated fatty acids from 47 to 51 g/100 g oil for red raspberry and 51 to 59 g/100 g oil for the blueberry seed flours (**Table 2**). These saturated fatty acid contents were different from that reported in their respective seed oils containing 2.3 and 8.6 g/100 g oil in the red raspberry and blueberry seed oils, respectively (*23*). Cranberry and chardonnay grape seed flours had fatty acid profiles similar to their previously reported respective seed oils (**Table 2**) (*37*−*39*). These results demonstrate that fruit seed flours may contain significant levels of oils, which may have different fatty acid profiles compared to their respective seed oils.

Oxygen Radical Absorbance Capacity. All tested fruit seed flours exhibited significant oxygen radical absorbing capacity with ORAC values of 110.5−1076 TE μmol/g (**Table 3**). The chardonnay grape seed flour had the highest ORAC among all tested fruit seed flours on a per weight basis. The ORAC value of the chardonnay grape seed flour was more than 3 times higher than that of the pinot noir grape flour and almost 10 times higher than that of the cranberry seed flour (**Table 3**). The current study determined the ORAC value of red raspberry and black raspberry seed flours to be 275.5 and 296.2 TE μmol/g, respectively (**Table 3**). These ORAC values were comparable but contradictory to that of 171 and 453 TE μmol/g reported for whole dried red raspberry and black raspberry, respectively (*32*). Also noted in this study was that the ORAC values of cranberry and blueberry seed flours were 110.5 and 152.9 TE μmol/g (**Table 3**), which were greater than the ORAC determined in the fresh cranberry and blueberry fruits (*4*, *6*). In 2003, Zheng and others reported ORAC values of 18.5 and 28.9 TE μmol/g for whole fresh cranberry and blueberry, respectively (*6*). Another study found the ORAC range of 9.6−26.0 TE μmol/g fresh fruit for blueberry cultivars (*4*). These data suggest that fruit seed flours are excellent dietary sources for oxygen radical absorbing components on a per weight basis. The ORAC values were correlated to TPC ($r = 0.992$, and $P = 0.01$),

Table 2. Fatty Acid (FA) Compositions of the Studied Cold-Pressed Seed Flours (g/100 g oil)[a]

FA	raspberry 1	raspberry 2	blueberry 1	blueberry 2	cranberry	pinot noir	chardonnay
12:0	nd	nd	1.3 ± 0.24	1.8 ± 0.12	t	0.8 ± 0.16	nd
14:0	1.5 ± 1.64	0.3 ± 0.01	0.1 ± 0.00	0.2 ± 0.00	t	0.4 ± 0.01	t
16:0	26.0 ± 0.58	28.0 ± 0.23	26.3 ± 0.95	29.9 ± 0.31	5.4 ± 0.01	35.0 ± 0.05	7.8 ± 1.07
16:1	2.0 ± 0.11	2.0 ± 0.15	0.4 ± 0.16	0.4 ± 0.08	0.1 ± 0.00	0.5 ± 0.01	0.2 ± 0.02
18:0	16.0 ± 0.23	18.8 ± 0.22	20.5 ± 0.53	25.7 ± 0.22	1.3 ± 0.00	2.7 ± 0.05	4.3 ± 0.57
18:1	34.1 ± 0.72	37.0 ± 0.02	46.1 ± 1.53	40.1 ± 0.53	25.1 ± 0.04	32.2 ± 0.05	19.2 ± 1.94
18:2	13.2 ± 0.23	8.0 ± 0.08	2.3 ± 0.50	0.1 ± 0.17	36.9 ± 0.05	13.0 ± 0.03	65.9 ± 3.95
18:3	1.9 ± 0.18	0.9 ± 0.13	nd	nd	30.9 ± 0.02	nd	1.8 ± 0.25
20:0	3.5 ± 0.06	3.8 ± 0.22	2.8 ± 2.33	1.5 ± 0.11	0.1 ± 0.01	1.3 ± 0.02	0.2 ± 0.03
20:1	1.8 ± 0.19	1.3 ± 0.11	0.3 ± 0.00	0.3 ± 0.00	0.2 ± 0.00	1.0 ± 0.02	0.5 ± 0.06
SFA	47.0	50.9	51.0	59.0	6.8	53.2	12.4
MUFA	37.9	40.3	46.7	40.8	25.4	33.7	19.8
PUFA	15.1	8.8	2.3	0.2	67.8	13.0	67.7
n-6/n-3	6.94	8.89	NA	NA	1.19	NA	36.61

[a] Data were expressed as mean ± SD ($n = 3$). Raspberry 1 and raspberry 2 are two different samples of the same variety of red raspberry. Blueberry 1 and 2 are different samples of the same variety. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids. n-6/n-3 is the ratio of n-6 to n-3 fatty acids. nd: not detected. t: trace.

Table 3. Antioxidant Activities of Selected Cold-Pressed Edible Seed Flours

	ORAC value [a]	$ED_{50-DPPH}$ [b]	chelating capacity[c]
black raspberry	296.2 ± 42.0 b	200	3.6 ± 0.20 a,b
red raspberry 1	275.5 ± 32.4 b	510	3.9 ± 0.07 a
blueberry 1	152.9 ± 27.3 c	670	1.9 ± 0.24 d
cranberry	110.5 ± 22.0 c	1260	2.1 ± 0.02 d
pinot noir grape	312.8 ± 16.5 b	160	2.6 ± 0.17 c
chardonnay grape	1076 ± 72.9 a	39	3.3 ± 0.14 b

[a] ORAC value is expressed as micromoles of Trolox equivalents (TE) per gram of seed flour (μmol/g flour). Values in the same column with different letters are significantly different ($P < 0.05$). [b] $ED_{50-DPPH}$ is the micrograms of flour equivalents per milliliter of reaction mixture (μg flour equiv/mL) to decrease the concentration of DPPH• to half of the initial concentration following 20 min of reaction. [c] Chelating capacity is expressed in EDTA equivalents milligram per gram of flour (EDTA equiv mg/g flour). Values in the same column with different letters are significantly different ($P < 0.05$).

suggesting that total phenolic content is a better indicator for oxygen radical absorbing components in the fruit seed flours.

DPPH•-Scavenging Activity. The chardonnay seed flour contained the highest level of DPPH• scavenging agents and had an $ED_{50-DPPH}$ value of 39 μg flour equivalents/mL (**Table 3**). The $ED_{50-DPPH}$ value is the concentration of a substance that will reduce the amount of DPPH• to half of the original concentration under the experimental conditions. The $ED_{50-DPPH}$ of the chardonnay seed flour was 4 times lower than that of pinot noir grape seed flour and 32 times lower than that for the cranberry seed flour (**Table 3**). In this study, 50% acetone was used for antioxidant extraction, because our previous study showed that 50% acetone extract of black raspberry seed flour quenched 14.9% more DPPH radicals than its 100% ethanol extract under the same testing conditions (*8*). Also, all fruit seed flour extracts demonstrated similar time and dose effects in their reactions with DPPH•, and the reactions of the pinot noir grape and red raspberry seed flour extracts are shown in **Figure 1**. The fruit seed flour extract with a stronger DPPH radical scavenging capacity also exhibited a higher ORAC value, but no significant correlation was detected. In addition, fruit seed flour extract with a stronger DPPH radical scavenging capacity had higher TPC, although no significant correlation was found.

Chelating Capacity. All fruit seed flour extracts demonstrated significant chelating capacities against Fe^{2+}. The values ranged from 1.9 to 3.9 EDTA equivalents mg/g flour (**Table 3**). The 50% acetone extract of red raspberry seed flour had the highest chelating capacity but was not significantly higher than that of the black raspberry seed flour. The ability of these fruit seed flours to coordinate metals may provide protection against metal ion induced free radical oxidation, because transition metals may act as catalysts to generate the first few radicals and initiate oxidative chain reactions in food and biological systems. It needs to be pointed out that these fruit seed flours

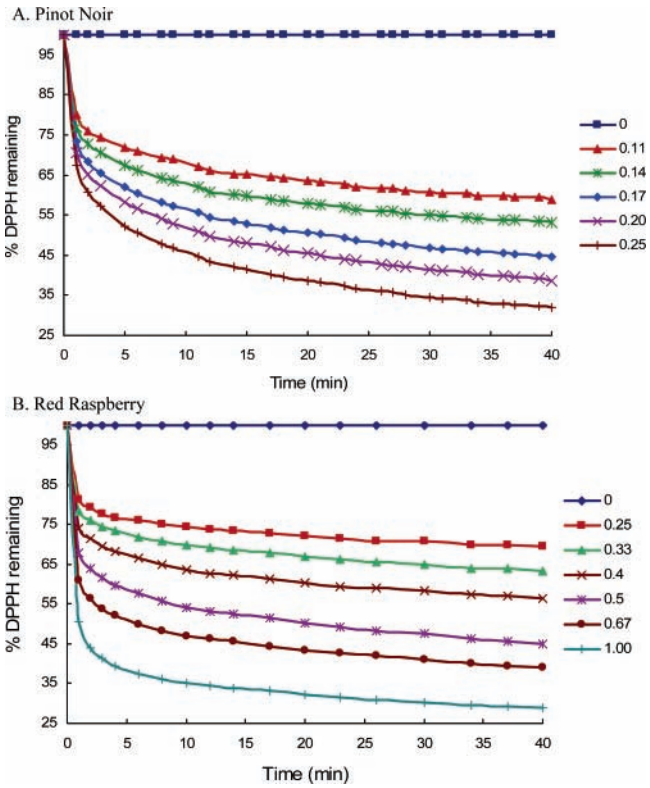

Figure 1. Dose and time effects of seed flour antioxidants–DPPH• reactions. The final concentration of DPPH radicals was 100 μM. (**A**) Reactions with pinot noir grape seed flour extract; 0.25, 0.20, 0.17, 0.14, 0.11, and 0 represent the concentrations of flour extracts in the initial reaction mixture at 0.25, 0.20, 0.17, 0.14, 0.11, and 0 mg seed flour equivalents per mL, respectively. (**B**) Reactions with red raspberry seed flour extracts extract; 1.00, 0.67, 0.50, 0.40, 0.33, 0.25, and 0 represent the concentrations of flour extracts in the initial reaction mixture at 1.00, 0.67, 0.50, 0.40, 0.33, 0.25, and 0 mg flour equivalents/mL.

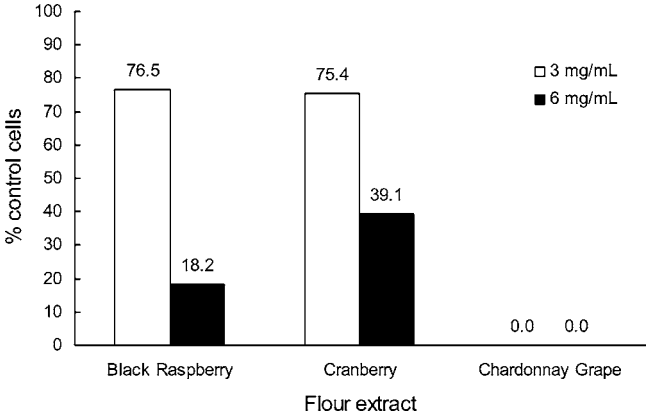

Figure 2. Proliferation rate of HT-29 colon cancer cells treated with the fruit seed extracts. Antiproliferative effects of the selected fruit seed flour extracts were expressed as percent control cells after exposure to treatment for 24 h. Black raspberry, cranberry, and chardonnay grape stand for the DMSO solutions of black raspberry, cranberry, and chardonnay grape seed flours prepared from the respective 50% acetone extracts, respectively. The final concentrations of for all fruit seed flour extracts were 3 and 6 mg flour equivalents/mL in the initial culture media.

may also interfere with essential mineral absorption due to chelating complex formation.

HT-29 Cell Proliferation. The DMSO solutions of chardonnay grape, black raspberry, and cranberry seed flours were evaluated for their potential anti-proliferative activities at dose levels of 3 and 6 mg seed flour equivalents per mL of culture media. These three extracts were selected because they represent the three genera involved in this study—*Rubus* (black raspberry and red raspberry), *Vaccinium* (cranberry and blueberry), and *Vitis* (pinot noit grape and chardonnay grape)—and the rest of the flour extracts were not tested due to the cost. Chardonnay grape seed flour extract completely eliminated all living HT-29 cells at both 3 and 6 mg flour equivalents/mL media following 24 h of exposure (**Figure 2**), whereas the extracts of black raspberry and cranberry dose-dependently suppressed cell proliferation under the same experimental conditions (**Figure 2**). Also noted was that the order of antiproliferative capacity against HT-29 cancer cells was same as that of TPC, but not as that of TAC, suggesting that total phenolic contents may be an important indicator for their antiproliferative activity. Both black raspberry and cranberry seed flour extracts inhibited the HT-29 cell proliferation in a dose-dependent manner through 4 days of experiments (**Figure 3**). These results indicate that these fruit seed flours contain different levels of antiproliferative components. To evaluate their potential utilization in cancer prevention, additional research is required to evaluate the antiproliferative activities of these fruit seed flour components on other cancer cells and normal cells, to investigate the underlying mechanisms, and to characterize the chemical structures contributing to the antiproliferation capacity.

In 2004, over 66 Mt of grapes were harvested globally (http://faostat.fao.org/faostat/), and the 2005 forecast for US production was 6.5 Mt with more than half of that projected for wine and juice production (http://www.nass.usda.gov). The total cranberry production in 2004 was 394 394 t (http://faostat.fao.org/faostat/), and over 70% was grown in the US (http://www.nass.usda.gov). From the US production, 99.9% of cranberries were processed. In addition, worldwide raspberry production was 461 485 t in 2004 (http://faostat.fao.org/faostat/). Red raspberry and black raspberry productions in the United States were 30 455 and 1000 t in 2004, and over 99.9% of each was processed (http://www.nass.usda.gov). Fruit seeds are byproducts from fruit

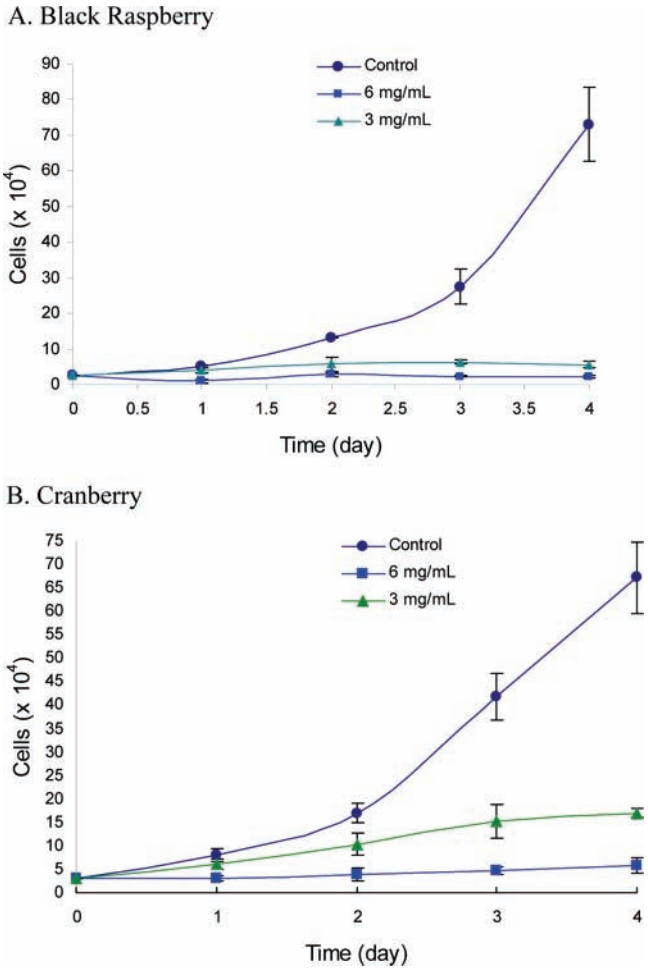

Figure 3. Dose and time effects of the selected fruit seed flour extracts on HT-29 cell growth. Black raspberry and cranberry represent the DMSO solutions of black raspberry and cranberry seed flours prepared from the respective 50% acetone extracts, respectively. The final concentrations of the fruit seed flour extracts were 3 and 6 mg flour equivalents/mL in the initial culture media. (**A**) The effect of black raspberry seed flour extract. (**B**) The effect of cranberry seed flour extract.

processing and may be used to obtain fruit seed oils with special fatty acid composition or other health beneficial components (*23*). Fruit seed flours are byproducts of fruit seed oil production and treated as low value wastes. Characterization of bioactive components in the fruit seed flours and demonstration of their potential beneficial properties may lead to value-added utilization of these fruit seed flours and enhance the profitability of the fruit production and processing industries and the fruit seed oil manufacturers. The results from this research suggest that these fruit seed flours may serve as dietary sources of natural antioxidants and contain antiproliferative components. Additional research is required to further investigate the effects of food formulation, processing, and storage on the availability of these beneficial components and properties, as well as the chemical and biochemical mechanisms involved in their antioxidant and antiproliferative properties in order to promote their utilization in food and dietary supplemental products for health promotion and disease prevention.

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