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ScienceDirect
Food Chemistry 104 (2007) 1065–1073
Food Chemistry
www.elsevier.com/locate/foodchem

Inhibitory effect of Chardonnay and black raspberry seed extracts on lipid oxidation in fish oil and their radical scavenging and antimicrobial properties

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Received 13 July 2006; received in revised form 18 December 2006; accepted 9 January 2007

Abstract

Ethanol extracts of Chardonnay grape and black raspberry seed flours were evaluated for their capacity to suppress lipid oxidation, preserve important fatty acids, and inhibit microbial growth. They were also tested for radical scavenging activity against DPPH and peroxyl radicals as reflected in oxygen radical absorbance capacity (ORAC), and total phenolic content (TPC). Both tested seed flour extracts suppressed lipid oxidation and rancidity development in fish oil. Black raspberry seed flour extract significantly reduced the degradation of biologically important $n-3$ PUFA under accelerated oxidative conditions. Black raspberry and Chardonnay seed flour extracts at 165 and 160 μg seed flour equivalents/mL, respectively exhibited bacteriocidal activity against *Escherichia coli* and growth inhibition of *Listeria monocytogenes* under experimental conditions. Both seed flour extracts exhibited DPPH radical quenching activity and Chardonnay had the stronger ORAC of 663 μmol Trolox equivalents per gram seed flour and the higher TPC of 99 mg gallic acid equivalents/g flour. The data from this study suggest the potential for developing natural food preservatives from these seed flours for improving food stability, quality, safety, and consumer acceptance.

Keywords: Fruit seed; OSI; Antimicrobial; Antioxidant; Radical scavenging; $n-3$ fatty acid; Phenolic; $ABTS^{\cdot+}$; $DPPH^{\cdot}$

1. Introduction

Lipid oxidation is a critical problem during food processing, distribution, storage, and consumption as it decreases food quality, stability, safety, and nutritive value. Antioxidants have been utilized to prevent oxidative damage to food components and prevent off-flavor development in food products (Yu, Scanlin, Wilson, & Schmidt, 2002). It is widely accepted that lipid oxidation in food products is a free radical mediated oxidative chain reaction involving three general phases: initiation, propagation, and termination. Antioxidants may suppress the initiation step and/or discontinue the propagation steps by reducing the availability of metal catalysts and quenching the radicals in the system, leading to the termination of oxidative radical chain reactions (Athukorala et al., 2003; Matthaus, 2002; Yu, Scanlin, et al., 2002). Antioxidants may also prevent the oxidation of protein in meat and other food products that contain high concentrations of prooxidants such as heme, transition metals and polyunsaturated fatty acids (Aligiannis et al., 2003; Viljanen, Kylli, Kivikari, & Heinonen, 2004). Recently, the demand for novel natural

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doi:10.1016/j.foodchem.2007.01.034

antioxidants has greatly increased, primarily due to the possible adverse side effects of synthetic antioxidants and the potential health beneficial effects of natural antioxidants (Aligiannis et al., 2003; Athukorala et al., 2003). A number of studies have been conducted to discover and develop natural antioxidants from agricultural products traditionally used for human consumption such as cereal grains, herbs, fruits, vegetables, marine red algae, and edible seeds and their fractions (Athukorala et al., 2003; Guleria, Vasudevan, Madhok, & Patwardhan, 1983; Ramadan, Kroh, & Morsel, 2003; Rey, Hopia, Kivikari, & Kahkonen, 2005; Velioglu, Mazza, Gao, & Oomah, 1998; Yu, Zhou, & Parry, 2005; Zhou, Su, & Yu, 2004). A few natural antioxidants such as rosemary extracts have been successfully developed for commercial utilization (Fernandez-Lopez, Zhi, Aleson-Carbonell, Perez-Alzarez, & Kuri, 2005; Yu, Scanlin, et al., 2002). Novel antioxidative preservatives with different physicochemical properties are needed for diversified food systems because the physical and chemical nature of a selected food system such as an emulsion or bulk oil may require an antioxidative preservative with different physicochemical properties (Frankel, Huang, & Prior, 1996).

It is also well recognized that food-borne pathogens are major concerns of food safety (Buzby, Roberts, & MacDonald, 1996). They are responsible for approximately 76 million cases of food-borne illness, 325,000 hospitalizations, and 5000 deaths in the United States annually (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5115a3.htm). The estimated annual cost related to the top five bacterial pathogens, including *Campylobacter, Salmonella* (nontyphoidal serotypes only), *Escherichia coli* 0157 and non-0157 STEC, and *Listeria monocytogenes*, is $6.9 billion. Grape pomace extract was shown to have antibacterial activity against thirteen different bacteria (Ozkan, Sagdic, Baydar, & Kurumahmutoglu, 2004). Antimicrobial activity of natural extracts is closely linked with their polyphenolic content (Ahn, Grun, & Mustapha, 2003). Therefore, grape and other fruit seed extracts rich in phenolics may serve as potential natural antimicrobial agents.

A recent study in our laboratory showed that 100% ethanol and 50% acetone extracts of black raspberry seed flours were able to directly react with and quench DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals (Parry & Yu, 2004). Phenolics were also detected in black raspberry seed flour extracts at a level of 26.7 and 45.6 mg gallic acid equivalents per gram of flour for ethanol and 50% acetone extracts, respectively (Parry & Yu, 2004). Furthermore, about 60–70% of the total extractable phenolic compounds in grapes are located in the seeds (Shi, Yu, Pohorly, & Kakuda, 2003). These data suggests the potential of fruit seed flour extracts in reducing lipid oxidation and the risk of foodborne illness caused by pathogens. Fruit seeds are readily available by-products from fruit processing, and seed flours are the by-products from seed oil production. The amount of caneberry seeds from Oregon and Washington seedless processing in 2003 alone was estimated 180,000 kg (Bushman et al., 2004). Developing natural food preservatives with both antioxidant and antimicrobial capacities from these fruit seeds may improve food quality, safety, and nutritional value for improving human health, while enhancing the profitability of fruit production and processing industries, as well as seed oil producers (Ahn et al., 2003; Fernandez-Lopez et al., 2005; Yu, Scanlin, et al., 2002).

The present study was conducted to evaluate the selected edible seed flour extracts for their potential to (1) inhibit lipid oxidation in fish oil; (2) preserve EPA and DHA in fish oil; (3) suppress bacterial growth; and (4) directly react with and quench DPPH radicals and absorb peroxyl radicals (ORAC). In addition, the total phenolic content and effect of these seed extracts on oil colour were examined. The data from this research may lead to novel natural food preservatives and improve the quality, safety, and nutritional value of food products.

2. Materials and methods

2.1. Materials and chemicals

Defatted Chardonnay grape (Ch, *Vitis vinifera*) and black raspberry (BR, *Rubus occidentalis*) seed flours were obtained from Botanical Oil Innovations, Inc. (Spooner, Wisconsin). Unstabilized menhaden fish oil was donated by Omega Protein (Reedville, VA) with manufacturer's specifications of an iodine value 170–200, a maximum peroxide value of 10 meq oxygen/kg, and a maximum of 0.50% free fatty acids. Mixed tocopherols, 2,2-diphenyl-1-picryhydrazyl radical (DPPH$^{\cdot}$), 2,2′-azinobis (2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), disodium ethylenediaminetetraacetate, and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO). Brain heart infusion agar (BHI) was purchased from Fischer Scientific (Difco, Detroit, MI) and bacteria were freezer stock strains of *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 19114. All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Preparation of antioxidant extract

Seed flours were ground to a #20 mesh using a Micromill manufactured by Bel-Art Products (Pequannock, NJ). Ten grams of ground seed flour were extracted with approximately 150 mL of 100% ethanol utilizing the Soxhlet extractor for 3 h. The ethanol extracts were kept in the dark under nitrogen at ambient temperature until further analysis. The concentration of each ethanol extract was calculated and expressed as the equivalents of starting material in mg per mL extract.

2.3. Oxidative stability index (OSI)

The stabilization of menhaden fish oil by the seed flour extracts was determined by OSI using a Rancimat

instrument (Model 743; Metrohm Ltd., Herisau, Switzerland). The oxidation was carried out with 6 mL fish oil containing different levels of seed flour extracts at 80 °C with an air flow of 7 L/h (Chen & Ho, 1997; Yu, Haley, Perret, & Harris, 2002). Two dose levels were tested for each antioxidant extract, and mixed tocopherols were used as the positive control. Fish oil containing no antioxidant was included as the negative control (the blank) to calculate the % extension time. Two separate doses, a low dose with 10% (v/v) and a high dose of 20% (v/v) extract in fish oil were combined and the ethanol was removed below 35 °C under reduced pressure using a rotary evaporator to obtain the testing oil sample. For the negative control (blank) an oil sample was combined with the same volume of ethanol and the ethanol was evaporated from the oil under the same experimental conditions. The low dose of antioxidants was 7.4 and 7.1 mg seed flour equivalent per mL of fish oil for Chardonnay grape and black raspberry seed flour extracts, respectively. The high dose of antioxidants was 16.7 and 16.0 mg seed flour equivalent per mL of fish oil for the Chardonnay grape and black raspberry seed flour extracts, respectively. Triplicate assays of the low dose and high dose and duplicate assays of the negative control were conducted. The results were expressed as the % extension time and calculated as:

$$\%\ \text{Extension time} = \frac{(\text{OSI}_{\text{sample}} - \text{OSI}_{\text{negative-control}})}{\text{OSI}_{\text{negative-control}}} \times 100\%,$$

where the OSI is defined as the hours required for an oil sample to develop measurably rancidity.

2.4. Fatty acid composition

Fish oil samples containing the antioxidant extracts were collected at 4.5 h of oxidation reaction time at 80 °C with an air flow rate of 7 L/h. These oil samples were analyzed and compared with the original fish oil (the blank) for their fatty acid composition. Relative concentrations of DHA, EPA, and total $n-3$ polyunsaturated fatty acids (PUFA) were of primary concern. Fatty acid methyl esters (FAME) were prepared from the oil samples and analyzed by gas chromatographic method (GC) according to a laboratory protocol (Yu, Adams, & Gabel, 2002). GC analysis was conducted using a Shimadzu GC-2010 with a FID and a Shimadzu AOC-20i autosampler (Shimadzu, Columbia, MD). A fused silica capillary column SP™-2380 (30 m × 0.25 mm with a 0.25 μm film thickness) from Supelco (Bellefonte, PA, USA) 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. Initial oven temperature was 142 °C and increased 6 °C/min to 184 °C and held for 3 min, then increased 6 °C/min to 244 °C. Identification of the individual fatty acids was accomplished by comparing GC retention time with that of fatty acid methyl ester standards. Quantification was based on the area under individual fatty acid peak and the total area of all fatty acid peaks. Results were reported as g fatty acid per 100 g total fatty acids. All samples were analyzed in triplicate.

2.5. DPPH radical scavenging activity

Free radical scavenging capacity of each seed flour extract was estimated following a previously reported procedure using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH·) (Yu, Scanlin, et al., 2002). Briefly, 800 μL of freshly made 200 μM DPPH· working solution was added in 800 μL of seed flour extract to start the radical-antioxidant reaction. The 200 μM DPPH· working solution was freshly prepared from a 1 mM DPPH· stock solution in ethanol and used within 8 h. The absorbance at 517 nm was determined against a blank of pure ethanol at 0.5, 1, 5, 10, 15 and 20 min of reaction and used to estimate the amount of DPPH radicals quenched. The initial concentration was 100 μM for DPPH·. The assay was conducted in triplicate.

2.6. Oxygen radical absorbance capacity (ORAC)

ORAC was estimated for 100% ethanol extracts of the seed flours using fluorescein (FL) as the fluorescent probe and a Victor multilabel plate reader (PerkinElmer, Turku, Finland) following an assay previously described (Moore et al., 2005). The trolox standard was dissolved in 100% ethanol while all other reagents were prepared in 75 mM phosphate buffer (pH 7.4). A final volume of 280 μL for each testing mixture was comprised of 225 μL of 8.16×10^{-8} M fluorescein (FL) solution, 30 μL of sample, standard or ethanol for the blank, and 25 μL of 0.36 M AAPH. The reaction mixture containing the FL and other agents was preheated for 20 min at 37 °C prior to the addition of AAPH solution. The fluorescence of each reaction mixture was recorded every minute for 45 min at 37 °C with the excitation and emission wavelengths at 485 nm and 535 nm, respectively. Trolox equivalents (TE) were calculated using the relative area under the curve for samples using a standard curve prepared using trolox under the same conditions. Results were expressed as μmoles of TE per g seed flours.

2.7. Total phenolic content (TPC)

The TPC of each seed flour extract was measured using Folin-Ciocalteu reagent according to a laboratory procedure described by Yu, Scanlin, et al. (2002). Briefly, the reaction mixture contained 100 μL of seed flour extract, 500 μL of the Folin-Ciocalteu reagent, 1.5 mL of 20% sodium carbonate, and 1.5 mL pure water. After 2 h of reaction at ambient temperature, absorbance was read at 765 nm and used to calculate the TPC using gallic acid as the standard. Triplicate reactions were conducted and results were reported as gallic acid equivalents (GAE).

2.8. Phenolic acid composition

After removing the solvent, the solid residues of each extract were redissolved in methanol, filtered through a 0.45 μm membrane and subjected to HPLC analysis of phenolic acid composition. Analysis was conducted using a Phenomenex C18 column (250 nm × 4.6 mm) according to a previously established protocol (Yilmaz & Toledo, 2004a). The phenolics were separated using a linear gradient elution program with solvents (A) 25% aqueous methanol in 1% acetic acid and (B) 75% aqueous methanol in 1% acetic acid with a sufficient wash time using (C) 96% methanol. Solvent ratios at a flow rate of 0.75 mL/min for the elution gradient were as follows: 1–30 min, 100% A; 3–45 min, 82% A and 18% B; 45–65 min, 72% A and 28% B; 65–75 min, 60% A and 40% B; 75–85 min, 40% A and 60% B; 80–90 min, 100% B; 90–110 min, 100% C. Duplicate samples of injection volume 20 μL were analyzed and identification was accomplished by comparing the retention time and spectrum of the peaks in the seed flour extracts to that of standard compounds. Analysis was conducted as 280 nm from 0–25 min and 360 nm thereafter. Quantification of an individual phenolic acid was conducted using the total area under each peak with the known external standard.

2.9. Antibacterial activity

Antibacterial activity of seed flour extracts was tested against *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 19114 according to established laboratory protocols (Zhao et al., 2001). *E. coli* was streaked on Luria-Bertani (LB; Difco, Cockeysville, MD) agar plate and *L. monocytogenes* on Brain Heart Infusion (BHI; Difco) agar plate from frozen culture stocks. A single colony was selected from each plate and subcultured on LB and BHI plates. Cultures were incubated at 35 °C for 24 h then resuspended in 5 mL of saline, and the optical density was adjusted to 0.09 (*E. coli*) and 0.06 (*L. monocytogenes*), which resulted in a bacterial concentration of approximately 10^8 CFU/mL. Bacteria suspensions were diluted 100 times with saline to obtain the working bacterial solution. A volume of 200 μL from each ethanol extract was mixed with 3.8 mL of bacteria cell suspension (10^6 CFU/mL) followed by 15 h of incubation at 35 °C (*E. coli*) or 4 °C (*L. monocytogenes*). The final concentration was 165 and 160 μg seed flour equivalent/mL for the Chardonnay grape and black raspberry seed flour extracts in the assay culture, respectively. Bacterial survival was measured by viable cell counting on LB agar for *E. coli* and BHI agar for *L. monocytogenes* after incubation using the serial dilution method (Marino, Bersani, & Comi, 2001). Triplicate assays were prepared for each flour extract. Equal volume of ethanol was used as the negative control. The antibacterial activity of the flour extracts was noted as the bacterial survival rates after incubation calculated as:

$$\text{Survival rate} = \frac{\text{Cell number in the treatment}}{\text{Cell number in the control}} \times 100\%.$$

2.10. Colour measurement

Ten mL fish oil containing low and high dose concentrations of each seed flour extract was analyzed for colour. Hunter colour values were obtained using a HunterLab Labscan spectrophotometer (Model 45/0; Reston, VA, USA) with a setting of D65/10° (daylight 65 illuminant/10° observer) (Yu, Scanlin, et al., 2002). Triplicate measurements were recorded for each concentration of the seed flour extract.

2.11. Statistical analysis

Data were reported as mean and standard deviation for triplicate measurements. Analysis of variance and Tukeys honestly significant difference tests were conducted (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) to determine differences among means. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

3.1. Oxidative stability index

Chardonnay grape and black raspberry seed oils are commercially available as specialty edible seed oils, and the seed flours from oil production are treated as wastes. Developing natural food preservatives from these edible seed flours may improve the profitability of the oil processing industry and benefit agriculture and food processing industries. Developing natural food preservatives from these edible seed flours may also improve food safety and consumer acceptability of food products. This study examined the potential of the selected seed flour extracts for their potential to inhibit lipid oxidation and preserve $n-3$ PUFA in fish oil. Oxidative stability index (OSI) measures the secondary products of lipid peroxidation by total volatile carbonyl compounds. OSI was defined as the time required for the oil to develop measurable rancidity under the experimental conditions. A larger OSI value is generally associated with a longer shelf life. With the Rancimat instrument oil samples are heated and air is pumped into the system leading to an acceleration of lipid peroxidation. This results in an elevated formation of volatile carbonyl compounds including aldehydes and small molecular weight carboxylic acids. The aldehydes may be further oxidized in air to carboxylic acids. These volatile compounds are collected in the pure water and increase the conductivity of the water as their concentration increases. Hence, the water conductivity is proportional to total volatile secondary products from lipid peroxidation, and reflects the degree of lipid peroxidation. Chardonnay and black raspberry seed flour extracts were able to suppress

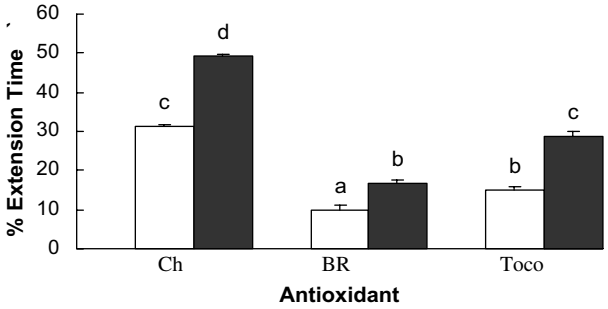

Fig. 1. Stabilization of fish oil by selected seed flour extracts. Ch, BR and Toco represent fish oil containing chardonnay grape and black raspberry seed flour extracts, or mixed tocopherols. Open bars represent the low dose of 7.41 mg/mL, 7.13 mg/mL, and 60 ppm for Ch, BR and Toco respectively. Solid bars represent the high dose with 16.68 mg/mL, 16.04 mg/mL, and 130 ppm for Ch, BR and Toco respectively. Data is expressed as means ± standard deviations ($n = 3$). Treatments sharing the same letter are not significantly different.

lipid oxidation and rancidity development in fish oil under the experimental conditions (Fig. 1). Chardonnay flour extract had the higher OSI of 2.85 h at 16.7 mg Chardonnay grape seed flour equivalents/mL, which represents an OSI extension of approximately 150% compared to the control. It was also noted that the Chardonnay grape seed flour extract at 7.4 mg flour equivalents/mL exhibited the same suppression of lipid oxidation in fish oil as 130 ppm mixed tocopherols (Fig. 1). These data suggest the potential of developing natural antioxidative preservatives from edible seeds and their fractions, especially Chardonnay grape seed flour. It needs to be pointed out that OSI is a measurement of oil stability under accelerated conditions, which may not truly reflect oil stability under common storage conditions.

It is important to note that ethanol was used as the extraction solvent in the present study, although our previous study showed that the 50% acetone extract of black raspberry seed flour had much stronger free radical scavenging ability and higher TPC content than that of its ethanol extract (Parry & Yu, 2004). Ethanol is a more practical extracting solvent for commercial scale production of antioxidative seed flour extracts. Ethanol may be reused, which reduces the environmental concern and overall cost on per unit of preservative basis. The solvent composition does not change during extraction and the extract quality and consistency may be better controlled.

3.2. Fatty acid composition

The extracts of Chardonnay grape and black raspberry seed flours were able to reduce the loss of eicosapentaenoic acid (EPA, C20:5$n-3$), 20:4$n-6$ (arachidonic acid), 18:3$n-3$ (α-linolenic acid), and total PUFA in the fish oil under the accelerated oxidation conditions (Table 1). Interestingly, black raspberry seed flour extract was more effective in preserving total $n-3$ fatty acids and PUFA than the Chardonnay grape seed flour extract, and maintained higher docosahexaenoic acid (DHA, 22:6$n-3$) content, although the grape seed flour extract exhibited stronger capacity in suppressing overall lipid peroxidation in fish oil under the experimental conditions (Table 1). Longer-chain $n-3$ polyunsaturated fatty acids DHA and EPA are well known for their potential in reducing the risk of heart disease, cancer, hypertension, and autoimmune disorders (Connor, 2000; Hung et al., 2000; Parry & Yu, 2004; Ruxton, Reed, Simpson, & Millington, 2004). Other PUFA such as linoleic and α-linolenic acids are also important for human health (Parker, Adams, Zhou, Harris, & Yu, 2003), and preservation of special PUFA is critical to maintain the nutritional value of the oil (Athukorala et al., 2003). The results from this study indicate that the capacity of an individual antioxidant preparation in preserving a selected fatty acid may differ to that in suppressing the overall lipid oxidation in the oil. The exact mechanism(s) underlying is not clear, but this observation may be partially explained by the different polarity of the antioxidants in each antioxidant preparations, which may lead to the different distribution of antioxidative components in the oil. This finding is important for creating optimal antioxidative food preservatives for minimized nutrient loss and rancidity development in different food systems.

3.3. DPPH· scavenging activity

To further understand the mechanisms involved in their antioxidative actions, these seed flour extracts were evaluated for their free radical scavenging capacity against DPPH and peroxyl radicals. Both seed flour extracts were able to directly react with and quench DPPH radicals (Fig. 2). Chardonnay grape seed flour extract exhibited the stronger DPPH· scavenging activity at 30 s of antioxidant-radical reactions. In contrast, after 5 min black raspberry seed flour extract showed the stronger DPPH· activity. These data indicate that these seed flour extracts have different kinetic and thermodynamic properties in their reactions with DPPH free radicals. Further research is required to analyze seed flour extracts for optimal concentrations and possible synergistic capabilities of combined seed flour extracts in different food models to scavenge free radicals and prevent spoilage.

3.4. Oxygen radical absorbance capacity (ORAC)

These extracts also exhibited significant oxygen radical absorbing capacities (ORAC) (Table 2). The Chardonnay grape seed flour extract demonstrated the highest ORAC value of 662 μmoles TE/g under the experimental conditions and the ORAC values from these seed flours were higher than that of whole caneberries (Wada & Ou, 2002). The results from this study were supported by previous findings demonstrating that caneberry seeds may have substantial quantities of tocopherols (Bushman et al., 2004), and grape seeds are widely recognized for their

Table 1
Preservation of PUFA in fish oil by selected seed flour extracts[A]

Fatty acid	Fatty acid composition (g/100 g fatty acid)				
	Unoxidized fish oil	Oxidized fish oil	Ch	BR	Toco
12:0	0.15a ± 0.01	0.17b ± 0.00	0.17ab ± 0.00	0.17b ± 0.01	0.16ab ± 0.01
14:0	11.58a ± 0.21	13.15c ± 0.05	12.90bc ± 0.02	12.58b ± 0.20	13.10c ± 0.02
14:1	0.05a ± 0.00	0.07b ± 0.00	0.06b ± 0.00b	0.06ab ± 0.00	0.06b ± 0.00
16:0	20.21a ± 0.01	22.45c ± 0.06	22.10c ± 0.03	21.57b ± 0.32	22.43c ± 0.11
16:1	15.64a ± 0.64	16.81b ± 0.03	16.32ab ± 0.01	16.15ab ± 0.19	16.75b ± 0.03
18:0	3.43a ± 0.07	3.88c ± 0.01	3.84bc ± 0.01	3.76b ± 0.04	3.93c ± 0.02
18:1	7.11a ± 0.39	8.38b ± 0.01	8.27b ± 0.01	8.16b ± 0.13	8.42b ± 0.04
18:2$n-6$	1.58a ± 0.00	1.64ab ± 0.01	1.80c ± 0.00	1.74bc ± 0.11	1.68abc ± 0.01
18:3$n-3$	1.67d ± 0.01	1.57a ± 0.00	1.64c ± 0.00	1.76e ± 0.01	1.61b ± 0.01
20:0	0.14d ± 0.00	0.16cd ± 0.00	0.16bc ± 0.00	0.15b ± 0.00	0.16d ± 0.00
20:1	1.18b ± 0.04	1.04a ± 0.00	1.04a ± 0.01	1.11ab ± 0.10	1.08ab ± 0.02
20:4$n-6$	1.24c ± 0.00	1.13a ± 0.01	1.22c ± 0.01	1.21bc ± 0.01	1.19b ± 0.02
20:5$n-3$	21.02c ± 0.03	18.38a ± 0.03	19.84b ± 0.03	19.55b ± 0.30	18.63a ± 0.09
22:6$n-3$	15.01b ± 0.03	11.17a ± 0.20	10.64a ± 0.06	12.02a ± 1.17	10.80a ± 0.30
Sat	35.50a ± 0.26	39.81d ± 0.12	39.16c ± 0.05	38.24b ± 0.55	39.78d ± 0.14
MUFA	23.98a ± 0.29	26.30c ± 0.04	25.70b ± 0.01	25.48b ± 0.21	26.31c ± 0.04
PUFA	40.52d ± 0.04	33.89a ± 0.15	35.14b ± 0.04	36.28c ± 0.76	33.90a ± 0.17
$n-3$	37.69c ± 0.04	31.12a ± 0.17	32.12a ± 0.03	33.33b ± 0.72	31.04a ± 0.16
$n-6$	2.82ab ± 0.00	2.77a ± 0.01	3.02c ± 0.01	2.95bc ± 0.10	2.87a ± 0.02
$n-6/n-3$	0.07a ± 0.00	0.09b ± 0.00	0.09b ± 0.00	0.09b ± 0.00	0.09b ± 0.00

[A] Data expressed as means ± standard deviations ($n = 3$). Values in the same row sharing the same letter are not significantly different ($P < 0.05$). Sat: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; $n-3$: $n-3$ PUFA; $n-6$: $n-6$ PUFA; $n-6/n-3$: ratio of polyunsaturated $n-6$ fatty acids to polyunsaturated $n-3$ fatty acids. Unoxidized fish oil: fresh menhaden fish oil, Oxidized fish oil: fish oil oxidized at 80 °C with an air flow rate of 7 L/h for 4.5 h without antioxidants, Ch and BR represent oxidized fish oil containing Chardonnay grape and black raspberry seed flour extracts at concentrations of 16.7 and 16.0 flour equivalents per mL oil, respectively. Toco represents oxidized fish oil samples containing mixed tocopherols at 130 ppm.

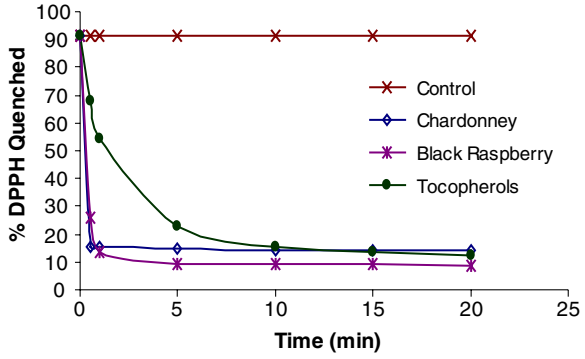

Fig. 2. DPPH Radical Scavenging Capacity of fruit seed flour extracts. The concentration of antioxidants was 26 mg seed flour equivalent/mL in the final antioxidant-radical reactions for all the tested seed flour extracts. Final concentration was 50 ppm for mixed tocopherols in the reaction mixture.

Table 2
Oxygen radical scavenging capacity and TPC[A]

Sample ID	ORAC (μmole TE/g)	TPC (mg GAE/g)
Chardonnay	662.5b ± 39.4	99.3b ± 3.7
Black raspberry	95.8a ± 4.5	11.8a ± 0.3

[A] Results for ORAC are expressed as μmoles trolox equivalent (TE) per gram of seed flour extract. Results for TPC are expressed as mg gallic acid equivalent (GAE) per g of seed flour. All data are reported as the mean ± the standard deviation ($n = 3$). Values marked by the same letter in the same column are not significantly different ($P < 0.05$).

antioxidant properties as well as their free radical scavenging abilities (Shi et al., 2003). Antioxidants may reduce the risk of cancer, cardiovascular disease, and dermal disorders (Pietta, 2000; Yilmaz & Toledo, 2004a). Grape skin and seed extracts are also safe for consumption in the human diet and may be suitable for antioxidant dietary supplementation (Yilmaz & Toledo, 2004b). Therefore, natural antioxidants may provide health benefits to consumers aside from improving food quality and stability.

3.5. Total phenolic contents

Total phenolic contents (TPC) were evaluated for the tested seed flour extracts because they contribute to the overall antioxidant activity. The TPC values of these seed flour extracts were in agreement with other radical scavenging and antioxidant tests (Table 2). Chardonnay seed flour had a TPC value of 99 mg gallic acid equivalents/g and was significantly higher than the black raspberry seed flour sample on a per flour weight basis, which may explain its ability to extend the shelf life of fish oil longer than the black raspberry seed flour extracts. The high TPC values of these flour extracts indicate their potential use as natural antioxidant ingredients to prevent oxidative rancidity and replace commonly used synthetic antioxidants such as butylated hydroxyltoluene (BHT) and butylated hydroxylanisole (BHA) (Singleton, Orthofer, & Lamuela-Raventos, 1999).

3.6. Phenolic acid composition

The seed flour extracts were tested for phenolic acid compositions. Similar to that previous reports on grape seed and skin, gallic acid, catechin, and epicatechin were found in grape seed flour at levels of 1.76, 7.70, and 1.30 mg/g seed flour on a dry weight basis, respectively (Yilmaz & Toledo, 2004a). In contrast, black raspberry seed flour only contained 0.31 mg ellagic acid/g seed flour on a dry weight basis. Yilmaz and Toledo (2004a) previously reported that flavanols, specifically catechin, were superior to phenolic acids in peroxyl radical scavenging capacities. The significant levels of catechin in the chardonnay seed flour extract may partially explain its very high in vitro antioxidant properties and capacity in prevention of lipid peroxidation in the fish oil.

3.7. Microbial growth inhibition assay

Extracts from botanicals have shown antimicrobial activity against various pathogenic microorganisms (Chorianopoulos et al., 2004; Singh, Kapoor, Pandey, Singh, & Singh, 2002). In the present study, the seed flour extracts were evaluated using *E. coli* and *L. monocytogenes* for their potential to inhibit the growth of food-borne pathogens. Both Chardonnay and black raspberry seed flour extracts at 3.3 and 3.2 mg seed flour/mL, respectively, exhibited bactericidal activity against *E. coli* with a zero survival rate. The extracts also had bactericidal activity against *L. monocytogenes* under specific experimental conditions, with a zero survival rate for the Chardonnay extract treatment. No bactericidal effect was found against *L. monocytogenes* under optimal culturing conditions indicating the limitation of the extracts (Table 3). In comparison, mixed tocopherols at a concentration of 25 ppm, which is equal to 25 μg α-tocopherol per mL, had no growth inhibitory activity against *L. monocytogenes* and had not bactericidal activity against *E. coli* as were the two seed flour extracts. These data suggest that these seed flour extracts have potential applications as natural food preservatives to inhibit microbial growth.

3.8. Colour evaluation

Colour is an important sensory property of food products. Many botanical extracts have a dark colour and may alter the visual perception of some final food products. The degree of colour alteration depends on the colour and level of the extract and the nature of the food matrix. Therefore, it is important to evaluate these fruit seed flour extracts in a food model for their possible effects on final colour. Effect of colour alteration by the addition of Chardonnay and black raspberry seed flour extract in oil was measured by the Hunter *L*-(lightness), *a*-(redness), *b*-(yellowness) values. Both Chardonnay and black raspberry flour extracts significantly influenced the "*L*", "*a*", and "*b*" values of the fish oil, primarily increasing the light and yellowness (Table 4). The black raspberry also signifi-

Table 3
Antibacterial activity against *E. coli* and *L. monocytogenes*[A]

Antioxidant	% Survival rate (Mean ± SD)		
	E. coli (35 °C)	*L. monocytogenes* (35 °C)	*L. monocytogenes* (4 °C)
Chardonnay	0.00a ± 0.00	Approx. 100a	0.00a ± 0.00
Black raspberry	0.00a ± 0.00	Approx. 100a	1.43b ± 0.38
Mixed tocopherol	3.12b ± 0.00	Approx. 100a	Approx. 100c

[A] Chardonnay grape and black raspberry seed flour extracts at concentrations of 3.3 and 3.2 mg seed flour equivalent/mL, respectively in the assay culture. Toco represents mixed tocopherols at 25 ppm (equivalent to about 1 mg α-tocopherol/mL). The antibacterial activity of seed meal extracts is noted as the bacterial survival rates after incubation. The lower the survival rate, the stronger the inhibition effect of the extract. Approximately 100% survival rate indicates that there was no inhibitory effect. Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Table 4
Effect of the tested seed flour extracts on oil colour

	Concentration (mg seed flour equivalent/mL oil)	*L*	*a*	*b*
Fish oil	–	3.55a ± 0.43	−1.92c ± 0.13	2.90a ± 0.55
Chardonnay	7.41	5.17c ± 0.04	−2.02c ± 0.12	4.57c ± 0.12
Chardonnay	16.68	4.57b ± 0.03	−1.18d ± 0.23	4.27c ± 0.17
Black raspberry	7.13	4.84c ± 0.08	−2.82b ± 0.18	4.07b ± 0.03
Black raspberry	16.04	6.39d ± 0.11	−3.63a ± 0.28	5.77c ± 0.21
Mixed tocopherol	60 ppm	3.10a ± 0.08	−2.03c ± 0.09	3.17a ± 0.18
Mixed tocopherol	130 ppm	3.07a ± 0.03	−2.00c ± 0.15	3.01a ± 0.18

Hunter colour values: '*L*' value measures lightness and varies from 100 for perfect white to zero for black; '*a*' value measures redness when positive, gray when zero, and greenness when negative; '*b*' value measures yellowness when positive, gray when zero, and blueness when negative. Data are expressed as means ± standard deviations ($n = 3$). Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

cantly increased the greenness. These data may be used to select the food product category to obtain the desired preservation with less undesirable colour alteration.

4. Conclusions

Results from this study suggest that natural food preservatives may be developed from edible fruit seed flours. These natural food preservatives may stabilize food products, maintain essential fatty acids, and reduce pathogenic microbial load to improve food quality, stability, nutritional value, and safety, while benefiting fruit producing and processing industries. Further characterization of their chemical composition is required to develop the commercial utilization of Chardonnay and black raspberry seed flours as commercial food preservatives. The results from this research also suggest the possibility of combining two or more natural extracts to maximize their effect in targeted food products.

Acknowledgement

This research was partially supported by a grant from USDA-CSREES National Research Initiatives, a federal Grant Number 20043550314852, the General Research Board Summer Award from the University of Maryland College Park, and a grant from the Maryland Agricultural Experimental Station.

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