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Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours

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ABSTRACT

Cold-pressed chardonnay, muscadine, ruby red, and concord grape seed oils and their defatted flours were studied for their fatty acid composition, oxidative stability and antioxidant and antiproliferative activities. The phenolic profiles of the seed flours were also measured. The most abundant fatty acid in the oils was linoleic acid, ranging from 66.0 g/100 g of total fatty acids in ruby red seed oil to 75.3 g/100 g of total fatty acids in concord seed oil. The oils were also high in oleic acid and low in saturated fat. Ruby red grape seed oil recorded the highest oxidative stability index of 40 h under the accelerated conditions. Total phenolic content (TPC) was up to 100 times lower in the oils than in the flours. Lutein, zeaxanthin, cryptoxanthin, β-carotene, and α-tocopherol levels were also measured. DPPH radical-scavenging capacity ranged from 0.07 to 2.22 mmol trolox equivalents (TE)/g of oil and 11.8 to 15.0 mmol TE/g of flour. Oxidative stability of menhaden fish oil containing extracts of the seed flours was extended by up to 137%. HPLC analysis was conducted to determine the levels of free soluble, soluble conjugated and insoluble bound phenolics in the seed flours. The phenolic compounds analyzed included catechin, epicatechin, epicatechin gallate, quercetin, gallic acid, and procyanidins B1 and B2. Antiproliferative activity was tested against HT-29 colon cancer cells. All of the seed flours and muscadine seed oil registered significant ($P < 0.05$) inhibition of cancer cell growth. The results from this study demonstrate the potential of developing value-added uses for these seed oils and flours as dietary sources of natural antioxidants and antiproliferative agents for optimal health.

1. Introduction

Grapes are mainly utilized for their juice, which is used primarily in wine-making. Grape seed oil is produced from the seeds in the pomace left over from juice and wine production, and thus adds value to the industry. This also helps to reduce waste disposal problems. Grape seed oil is gaining popularity as a culinary oil, and has been studied as a possible source of specialty lipids. It is a rich source of linoleic acid (Beveridge, Girard, Kopp, & Drover, 2005), which is associated with promotion of cardiovascular health by down-regulating low-density lipoprotein cholesterol (LDL-C) production, and enhancing its clearance (Wijendran & Hayes, 2004). Cold-pressing is a method of oil extraction that involves no heat or chemical treatment, and hence may retain more health beneficial components, such as natural antioxidants (Yu, Zhou, & Parry, 2005). The cold-pressed oils may be a better source of beneficial components, such as antioxidative phenolic compounds (Bail, Stuebiger, Krist, Unterweger, & Buchbauer, 2008), as well as other health-beneficial phytochemicals (Crews et al., 2006). Although the yield is usually lower than that with conventional solvent extraction, there is no concern about solvent residues in the oil, making for a safer and more consumer-desired product.

Grape seed flour, the residue from seed oil manufacture, has not received much attention but may be a potential rich source of natural antioxidants and other healthful bioactive compounds (Luther et al., 2007). For instance, grape seed proanthocyanidins were found to induce apoptosis and inhibit metastasis in both cultured breast and colon cancer cells (Mantena, Baliga, & Katiyar, 2006). In 2007, our research group reported that an ethanol extract of chardonnay grape seed flour not only suppressed overall lipid peroxidation in fish oil, but also protected eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the longer bioactive n-3 fatty acids, against oxidative loss (Luther et al., 2007). These findings were supported by the observations of a recent finding that grape seed residues from mechanical oil extraction were rich in polyphenolics with strong antioxidant properties (Maier, Schieber, Kammaerer, & Carle, 2009). These previous studies suggested the potential of developing natural antioxidative preparations from grape seed flours for shelf-life enhancing applications in foods.

Lipid peroxidation is a major problem of food quality, safety and nutritional value. Lipid peroxidation leads to off-flavour

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development in foods and reduces the nutritional and economic value of food products. Antioxidants are often added to food formulae to increase their oxidative stability and prolong shelf life. Many of the antioxidants used in the food industry are chemically synthesised: mainly butylated hydroxyl anisole, butylated hydroxyl toluene (BHA and BHT, respectively) and propyl gallate (Singh, Marimuthu, de Heluani, & Catalan, 2005). Natural antioxidants derived from edible materials are in high demand for food applications amidst concerns over the safety of the consumption of these synthetic antioxidants (Ito, Fukushim, & Tsuda, 1985).

As part of our ongoing efforts to develop value-added utilisations of fruit seeds, this study was conducted to determine: (1) the fatty acid profile, oxidative stability, and phytochemical composition of the selected cold-pressed grape seed oils; (2) the soluble free, soluble conjugated and insoluble bound phytochemical compositions and the antioxidant properties of the grape seed flours; (3) the antiproliferative activity of the seed oil and flour extracts against HT-29 colon cancer cells.

2. Materials and methods

2.1. Materials and reagents

Cold-pressed chardonnay, concord, muscadine, and ruby red grape seed oils and their defatted flours were obtained from Botanical Oil Innovations Inc. (Spooner, Wisconsin). Unstabilised and stabilized menhaden fish oils were donated by Omega Protein (Reedville, VA). Gallic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were purchased from Sigma–Aldrich (St. Louis, MO). Methanolic HCl (3 N) was obtained from Supelco (Bellefonte, PA). All components of the cell culture media were purchased from Invitrogen (Carlsbad, CA), except biological grade dimethyl sulfoxide, which came from Sigma–Aldrich (St Louis, MO). All other chemicals and solvents were of the highest commercial grade and were used without further purification.

2.2. Preparation of antioxidant extract from defatted grape seed flours

The seed flours were ground to mesh size 20. The Soxhlet method was used to extract 10 g of each of the ground seed flours with 100% ethanol for 3 h (Luther et al., 2007). The final volume of each extract was made up to 150 ml. The extracts were stored under nitrogen in the dark prior to further analysis.

2.3. Preparation of antioxidant extract from grape seed oils

One gramme of each cold-pressed seed oil was extracted with 3 ml of methanol (1 ml × 3 times) according to a laboratory procedure (Parry, Hao et al., 2006). The methanol extract for each oil was kept under nitrogen in the dark at ambient temperature prior to further analysis.

2.4. Fatty acid composition of oils

Fatty acid methyl esters (FAMEs) were prepared according to a laboratory protocol described previously (Parry et al., 2005). Briefly, 1 mg of oil was reacted with 0.1 M NaOH—MeOH for 5 min followed by reacting with 4% HCl—MeOH for 5 min, at ambient temperature. After adding water to stop the reaction, FAMEs were extracted with iso-octane. GC analysis was conducted with a Shimadzu GC-2010 equipped with a FID and a Shimadzu AOC-20Si 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) 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 temperature was 142 °C; it was increased at 6 °C/min to 184 °C, held for 3 min, and then increased at 6 °C/min to 244 °C. Individual fatty acid methyl esters were identified by comparing their retention times with those of FAME standards. Area under each fatty acid peak relative to the total area of all fatty acid peaks was used to quantify the fatty acids identified. Results are reported as g fatty acid/100 g of total fatty acids. All samples were analysed in triplicate.

2.5. Carotenoid composition and α-tocopherol content

The seed oils and flours were analysed for their contents of α-tocopherol and four different carotenoids (lutein, zeaxanthin, β-carotene, and β-cryptoxanthin) according to the protocol described by Hao, Parker, Knapp, and Yu (2005). Briefly, oil samples were mixed with hexane, and flour samples were extracted with hexane and centrifuged to obtain the testing samples, which were then subjected to LC–MS analysis. A TSQ quantum tandem mass spectrometer (Thermo-Finnigan, San Jose, CA), equipped with an ESI interface, and an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) with a Zorbax SB C18 column, 50 × 1.0 mm i.d., with a 3.5 μm particle size (Agilent Technologies), was used to separate the carotenoid compounds. Identification of the individual components was accomplished by comparing HPLC retention time and selected reactant monitoring (SRM) analyses of the sample peaks with that of the authorised pure individual commercial compounds. Quantifications for the carotenoid and tocopherol compounds were conducted using the total ion counts with an external standard. Data were obtained using Xcalibur software (Thermo-Finnigan). Quantification for α-tocopherol and each carotenoid was done using the total ion counts of the corresponding external standards.

2.6. Total phenolic content (TPC)

The TPC values of the seed oil and flour extracts were measured using the Folin–Ciocalteu reagent, according to a previously described laboratory protocol (Yu, Perret, Harris, Wilson, & Haley, 2003). Briefly, the reaction mixture contained 100 μl of antioxidant extract or solvent, 500 μl of the Folin–Ciocalteu reagent, 1.5 ml of 20% sodium carbonate, and 1.5 ml of 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 measurements were taken, and results were expressed as mg gallic acid equivalents (GAE)/g of seed oil or flour.

2.7. Relative DPPH·-scavenging capacity (RDSC)

Radical-scavenging capacity was assessed against the stable 2,2-diphenyl-1-picryhydrazyl radical. Antioxidant extracts of oil and flour samples were evaporated in the rotary evaporator and quantitatively re-dissolved in 50% acetone. RDSC values were obtained using a high throughput laboratory assay (Cheng, Moore, & Yu, 2006). Measurements were carried out on a Victor^3 Multilabel Plate Reader (PerkinElmer) with 96-well plates. The reaction mixtures contained 100 μl of 0.2 mM DPPH· and 100 μl of solvent for the control, standard, or sample. Absorbance was read at 515 nm every minute for 40 min. Trolox was used as the standard, and five different concentrations were used to plot the standard curve. Determinations were carried out in triplicate.

2.8. Oxidative stability index (OSI)

Oxidative stability was determined with a Rancimat instrument (Model 743; Metrohm Ltd., Herisau, Switzerland). Oxidation was