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Antioxidants and bioactivities of free, esterified and insoluble-bound phenolics from berry seed meals

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ABSTRACT

Phenolic compounds present in the free, soluble ester and insoluble-bound forms of blackberry, black raspberry and blueberry were identified and quantified using high-performance liquid chromatography-diode array detection-electrospray ionisation multistage mass spectrometry. The total phenolics, scavenging activity against hydroxyl and peroxyl radicals, the reducing power and chelating capacity were, in general, in the decreasing order of blackberry > black raspberry > blueberry. Amongst fractions, the order was insoluble-bound > esterified > free. These trends were the same as those found against copper-induced LDL-cholesterol oxidation and supercoiled plasmid DNA strand breakage inhibition induced by both peroxyl and hydroxyl radicals. Extracts were found to contain various levels of phenolic compounds that were specific to each berry seed meal type. Berry seed meals should be considered as a good source of phenolics with potential health benefits. Their full exploitation may be helpful for the food industry and consumers.

1. Introduction

The consumption of fresh fruits and vegetables has continued to increase, primarily due to a better understanding about the association of their intake with a lower incidence of cardiovascular disease, cancer, degenerative diseases, and other chronic ailments. Oxidative stress, which is induced by free radical attack on cellular components by reactive oxygen species (ROS), has a major role in the development of many degenerative diseases. As a result, antioxidants protect against oxidative stress and, therefore, they are considered important in reducing the initiation and progression of these diseases. Endogenous antioxidant systems play a crucial function in combating oxidative stress, but dietary antioxidants are also important (Cotran, Kumar, & Collins, 1999; Shahidi & Ambigaipalan, 2015).

Fruits and vegetables are valuable sources of phytochemicals, such as carotenoids, vitamins C and E, folate, phenolic and thiol compounds. The relationship between increased fruit and vegetable intake and lower risk of chronic diseases could be attributed to their antioxidant activity. Unlike animal dietary sources, plant-based diets contain many simple phenolic and polyphenolic compounds that possess significant antioxidant properties. In plants, the polyphenolic compounds provide several eco-physiological functions, involving chemical and physiological defense responses, and they are varied in their structures and chemical properties. As components in the human diet, phenolic compounds are considered to act as antioxidants directly, or to affect the production or function of other antioxidant compounds in the body (Halvorsen et al., 2002; Prior, 2014).

Antioxidant efficiency may be evaluated by several methods, which stems from their capacity in scavenging radicals by single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms (Leopoldini, Marino, Russo, & Toscano, 2004). These assays vary in terms of their principles and experimental conditions. Therefore, each assay provides an estimate of antioxidant efficiency that is based on the type of method selected and experimental conditions employed. Thus, the use of different methods assists in identifying variations in the compounds extracted from plant sources.

In the production of juices, jellies and jams from berries, most processors remove the berry seeds prior to processing. The seeds are commonly used as a source of specialty oil and the leftover meal (flour) is rich in bioactive ingredients (Helbig, Böhm, Wagner, Schubert, & Jahreis, 2008). Unlike oil seeds, berry seed meals have not commonly gained much attention as antioxidant sources, this could be attributed to their lack of popularity and varied commercial applications. Thus, it would be beneficial, if they could be employed as a source of natural food additives and ingredients in enhancing the complete utilisation of the seeds.

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Therefore, the aims of this study were to determine the total phenolics of selected berry seed meals, identify the various phenolic compounds in each sample by high performance liquid chromatography-diode array detection-electrospray ionisation-tandem mass spectrometry (HPLC-DAD-ESI-MS^n) and evaluate the potential health benefits of these byproducts to deactivate ROS and metal ions, followed by testing of their efficacy in biological model systems.

2. Materials and methods

2.1. General

The seed meal of blackberry, black raspberry, and blueberry samples were provided by the Fruit Smart Company, Grandview, WA, USA. Samples were packed immediately in vacuum bags and stored in a freezer at −20 °C.

Trolox(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Acros Organics (Fair Lawn, NJ, USA). Organic solvents and reagents, such as methanol, acetone, acetonitrile, formic acid, hydrochloric acid, sodium hydroxide, and sodium carbonate, were purchased from Fisher Scientific Co. (Nepean, ON, Canada). 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), Folin and Ciocalteau's phenol reagent, and all phenolic compound standards with a purity of ⩾96%, sodium hydroxide, hydrogen peroxide, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), ferrous sulphate, fluorescein, potassium ferricyanide, trichloroacetic acid, ferrous chloride, ferrozine, ferric chloride, human LDL-cholesterol, $CuSO_4$, sodium chloride, mono- and dibasic sodium and potassium phosphates, ethylenediaminetetraacetic acid (EDTA), deoxyribonucleic acid (DNA) of pBR 322 (*Escherichia coli* strain RRI), agarose, Tris acetate, bromophenol blue, xylene cyanol, and glycerol were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada).

2.2. Preparation of samples

The oil from the berry seed meals were commercially removed but there are still some residual oil remaining in the berry seed meals. Therefore, an extra effort is needed to remove them from the samples. Thus, all samples were defatted by blending the ground material with hexane (1:5, w/v, 5 min) in a Waring blender (Model 33BL73, Waring Products Division, Dynamic Corp of America, New Hartford, CT, USA) at room temperature (24 °C), three times. The resultant extracts were combined and filtered through Whatman #1 filter paper with suction using a Buchner funnel. Defatted samples were air dried for 12 h and vacuum packaged in polyethylene pouches and kept at −20 °C until used.

2.3. Extraction of phenolics

Extraction of phenolic acids and minor amounts of flavonoids, proanthocyanidins and anthocyanins from the defatted berry seed meals were performed according to the method outlined by Krygier, Sosulski, and Hogge (1982), and as explained by Naczk and Shahidi (1989). Soluble phenolics were extracted using methanol–acetone–water (7:7:6, v/v/v), acetone–water (80: 20, v/v), methanol–water (70:30, v/v), or water, and each was used separately. About 5 g of each sample were extracted with 75 ml of each solvents. These samples were ultrasonicated for 20 min at 30 °C, then centrifuged for 5 min at 4000*g*. After centrifugation, the upper layers were combined and the extraction operation was repeated twice. The collected supernatants were examined for free phenolics and soluble phenolic esters, and the solid residue was stored for evaluation of insoluble-bound phenolics. The organic solvent from combined extracts was removed under vacuum at 40 °C using a rotary evaporator (Rotavapor model 461, Büchi, Flawil, Switzerland). Before extraction with hexane, the aqueous phase was acidified to pH 2 using 3 M HCl and centrifuged to separate any precipitates. Extraction of free phenolics was carried out 3 times with an equal volume of diethyl ether–ethyl acetate (1:1, v/v). Diethyl ether–ethyl acetate layer was filtered through anhydrous sodium sulphate, using a No. 1 Whatman filter paper, and combined, evaporated to dryness, and dissolved into 5 ml of HPLC grade methanol. The esters present in the aqueous phase were hydrolysed with 4 M NaOH for 4 h. The pH of the hydrolysate was adjusted to 2 and the liberated phenolics were extracted with diethyl ether–ethyl acetate (1:1, v/v), evaporated to dryness and subsequently dissolved in 5 ml methanol. The solid residue obtained following the extraction of the soluble phenolics was dissolved in 40 ml of 4 M NaOH whilst stirring for 4 h. The mixture was then adjusted to pH 2, centrifuged and the insoluble-bound phenolics were extracted with diethyl ether–ethyl acetate (1:1, v/v) in the same manner as explained above.

2.4. Determination of total phenolic content

The total phenolics were estimated according to the method of Singleton and Rossi (1965). The Folin and Ciocalteau's phenol reagent (0.5 ml) was mixed with 0.5 ml of methanolic extracts in a centrifuge tube, and 1 ml of saturated sodium carbonate (75 g/L) was added to each tube, followed by adjusting the volume to 10 ml with distilled water. The contents were allowed to stand for 45 min at ambient temperature (23 °C). The absorbance was read at 725 nm. The total amount of phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per gram of defatted sample.

2.5. HPLC-DAD-ESI-MS^n analysis

In order to identify the predominant phenolic compounds in the berry seed meal samples, high performance liquid chromatography-diode array detection-electrospray ionisation multistage mass spectrometry (HPLC-DAD-ESI-MS^n) was used (Agilent Technologies, Palo Alto, CA, USA). A slightly modified version of the method described by Määttä, Kamal-Eldin, and Törrönen (2003) and Wu and Prior (2005) was used. Analytical separation of the phenolic compounds was carried out on a Supelcosil LC-18 column (250 × 4.6 mm inner diameter, 5-μm particles, Supelco, Bellefonte, PA, USA). The column oven temperature was maintained at 25 °C. Mobile phases consisted of formic acid (A) and acidified water containing 0.1 M acetonitrile (B). The elution conditions were as follows: 0 min, 100% B; 5 min, 90% B; 35 min, 85% B; 45 min, 60% B; 50 min, 60% B; 55 min, 100% B; 65 min, 100% B and then held for 1 min before returning to the initial conditions. The flow rate was 0.5 ml/min and the wavelengths of detection were 280 and 520 nm. Peak identification of unknown compounds of interest in this study was performed by matching the retention times of unknown compounds with external standards, when available. HPLC-ESI-MS^n analysis was carried out as described by de Camargo, Regitano-d'Arce, Biasoto, and Shahidi (2014a) under the same conditions as described above using an Agilent 1100 series capillary liquid chromatography/mass selective detector (LC/MSD) ion trap system in electrospray ionisation (ESI) in the negative and positive mode. The data were acquired and analysed with an Agilent LC/MSD software. The scan range was set from 50 to 1200 m/z, using smart parameter setting, drying nitrogen gas at 350 °C, flow 12 L/min, and nebulizer gas pressure of 70 psi.

2.6. Hydroxyl radical scavenging activity

The capacity of phenolic compounds in scavenging hydroxyl radicals formed by Fenton reaction was examined by electron paramagnetic resonance (EPR) spectroscopy, using the method