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Antioxidant and quinone reductase inducing activities of ethanolic fractions from purple maize

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ABSTRACT

Ethanolic extracts of three different varieties of purple maize (*Zea mays* L.) were examined for scavenging activities toward nitric oxide (NO•) and superoxide ($\cdot O_2^-$) and the ability to induce quinone reductase (QR). The crude extracts showed a dose-dependent antioxidant activity in the following order: generic purple > Oaxaca 332 > Veracruz 42. The extract of the generic purple variety exhibited the highest ability to induce QR activity and antioxidant activity was split into several fractions that showed antioxidant activities and capacity for doubling QR specific activity. Fraction III doubled QR at a content of 105 μg/mL (CD value). Semi-preparative LC and UV spectral and MS analyses of fraction III indicated the presence of glucosidic forms of cyanidin, peonidin, pelargonidin and their respective acylated counterparts.

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1. Introduction

The human body is constantly exposed to potential carcinogens in the environment; induction of phase II detoxification enzymes by dietary components is a potential cancer chemopreventive strategy (Wilkinson & Clapper, 1997). These enzymes are responsible for degrading reactive electrophilic intermediates through conjugation and reduction reactions, thereby protecting cells from oxidative DNA damage. The phase II enzyme quinone reductase (QR) catalyzes two-electron reductions of toxic oxygen metabolites, facilitating the deactivation of these reactive oxygen species and protection of tissues from oxidative damage. QR also serves as a biomarker for up-regulation of a suite of phase II enzymes and other cytoprotective elements that help cells maintain redox homeostasis and defend against inflammatory processes (Chen & Kunsch, 2004). Previous studies have shown many dietary phytochemicals to stimulate expression of phase II enzymes in cells and tissues (Fahey & Stephenson, 2002; Wattenberg, Sparnins, & Barany, 1989; Zhang, Talalay, Cho & Posner, 1992). Various flavonoids and phenolics compounds are among the most commonly identified phase II enzymes inducers.

Extracts from edible fruit and vegetable tissues have also been shown to induce phase II enzymes (González-Montilla, Chávez-Santoscoy, Gutiérrez-Uribe, & Serna-Saldivar, 2012; Lopez-Martinez, Parkin, & Garcia, 2011). In some cases, the QR induction bioassay-directed isolation has led to identification of the active components in crude extracts (Kang & Pezzuto, 2003; Zhang et al., 1992). Some crude extracts of maize kernel extracts were found to be among the most potent of a series of vegetables examined in preliminary studies (Wettasinghe, Bolling, Plhak & Parkin, 2002). Maize (*Zea mays* L) is the most domesticated plant on the planet; there are several pigmented types of maize, which are included in 41 races in México (Ortega, Sanchez Castillo & Gonzalez, 1991). Colors as red, black or purple are attributed to anthocyanins which are present in pericarp, aleurone or in both grain structures.

Extensive studies indicate that anthocyanins have strong free radical scavenging and antioxidant activities (Li et al., 2012; Lopez-Martinez et al., 2009), and suggest that anthocyanins play an important role in preventing mutagenesis and carcinogenesis. Caillet et al. (2012) reported that juice and extracts from cranberries were effective inducers of QR activity at an optimal concentration of 25 mg/mL Lopez-Martinez et al. (2011), showed the effectiveness of purple maize in terms of inducing protective phase II enzymes in murine hepatoma cells.

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The general aim of this study was to evaluate nitric oxide and superoxide scavenging activities and QR-inducing activity in kernel ethanolic extracts of three purple maize strains. This information will be useful to the food industry to promote the use of purple maize extracts as a possible food ingredient of natural origin and a source of health-promoting compounds.

2. Materials and methods

2.1. Materials

The maize samples of varieties Oaxaca 332 and Veracruz 42, were provided by Campo Cotaxtla (INIFAP), near the city of Veracruz, and a generic purple maize was obtained in a local market in the State of México. All chemicals, biochemicals and Sephadex LH-20 were purchased from Sigma–Aldrich Co. (St. Louis, MO); acetic acid, ethanol, methanol, and HPLC-grade acetonitrile were purchased from Fisher Scientific (Chicago, IL). Costar microtiter plates were obtained from Corning Inc. (Corning, NY). Fetal bovine serum (FBS), antibiotics and α-minimum essential medium (MEM) were purchased from Gibco Life Technologies (Grand Island, NY).

Maize kernels were ground in a cyclone mill (UDY, Boulder, CO) to pass through 250 μm screen mesh before extraction. The Folin-Ciocalteu method was employed to estimate phenolics content according to Gao, Wang, Oomah, and Mazza (2002), while total contents of anthocyanins were determined using the pH differential method (Giusti & Wrolstad, 2001).

2.2. Preparation of extracts

Ca. 35 g of ground maize kernels were refluxed with 420 mL of 95% ethanol in a Soxhlet extraction apparatus for 3 h at 78 °C. The resulting ethanolic extract was filtered and the solvent was evaporated using a rotary evaporator (Büchi, Flawil, Switzerland) at 40 °C. The concentrate was collected and freeze-dried for 72 h at 13.3 Pa, then stored in amber glass vials at 4 °C in the dark, until used.

2.3. Fractionation of phenolic compounds

Dry matter from the crude ethanolic extract (300 mg) was dissolved in 5 mL of 50% (v/v) aqueous methanol and was applied to an activated Oasis HLB cartridge (Waters Corp., Milford, MA). The cartridge was washed with 10 mL of 15% methanol to remove the phenolic acids and then with 15 mL of acidified methanol (5% formic acid in methanol) to elute anthocyanins. The anthocyanin fraction was applied to a second Oasis HLC cartridge that was washed with 10 mL of 5% formic acid, followed by 15 mL of ethyl acetate and then 10 mL of 10% formic acid in methanol. Ethyl acetate eluted the flavonoids and the acidified methanol eluted the anthocyanins. The anthocyanin fraction was collected and concentrated in a rotary evaporator to remove the solvent at 40 °C and the isolates were freeze-dried for 72 h at 13.3 Pa.

500 mg of the anthocyanin fraction were dissolved in 3 mL of 90% (v/v) aqueous ethanol; this solution was loaded onto a Sephadex LH-20 column (2.5 cm × 75 cm, particle size 25–100 μm, Sigma Chemical, Co., Nepean, ON) and eluted with 90% (v/v) aqueous ethanol. Elution of organic material was monitored by absorbance at 520 nm to allow for pooling of collected material. Ethanol was evaporated from the pooled fractions under vacuum at 50 °C using a rotary evaporator, and the isolates were freeze-dried for 72 h at 13.3 Pa. Extraction and fractionation were repeated five times, and the fractions were pooled together to obtain sufficient amounts for the bioassay.

2.4. Liquid chromatography analysis

The fraction with the highest activity was analyzed by HPLC (Agilent model 110 system, Wilmington, DE), fitted with a photodiode array detector. The mobile phase consisted of an acetonitrile:water (both solvents contained 2% acetic acid) gradient of 5:95 to 40:60 over the first 25 min, to 80:20 over 2 min with an additional 10 min hold, followed by 5 min post-run time with HPLC-grade water. A reverse phase column (Discovery C18, 25 cm × 4.6 mm, 5 μm particle size, Supelco, Bellefonte, CA) was employed. Active fractions were further resolved by semi-preparative HPLC (Discovery C18 column, 25 cm × 10 mm, 5 μm particle size, Supelco, Bellefonte, CA) monitored by UV detection at 520 nm with simultaneous peak integration (SP2479 Integrator, Spectra Physics, San Jose, CA). A gradient of acetonitrile:water in 1% acetic acid was employed, with an initial hold at 5:95 for 9 min, ramped to 12:88 over 24 min, then ramped to 80:20 over 2 min and held at 80:20 for an additional 24 min, using a flow rate of 3 mL/min. Peaks were obtained as different sub-fractions at 520 nm. Solvents were evaporated under vacuum at 50 °C with a rotary evaporator and the resulting aqueous material was freeze-dried for 72 h at 13.3 Pa, then stored in amber glass vials at 4 °C in the dark until used. Low-resolution electrospray mass spectrometry was performed with an Esquire 3000 ion trap mass spectrometry (MS) (Bruker Daltonik, Billerica, MA) with a Surveyor autosampler, diode array detector and HPLC (Thermo Electron, San Jose, CA) equipped with an electrospray ionization (ESI) source. Briefly, a 5 μL sample was injected onto a 250 × 2 mm Synergi Hydro RP column (Phenomenex, Torrance, CA) equilibrated in 95:5 A:B where A = 1% formic acid in water; B = 1% formic acid in acetonitrile, flow rate = 0.23 mL/min. Anthocyanins were eluted through application of a linear gradient to 75:25 A:B in 50 min. The experimental conditions were: ESI interface, nebulizer, 45 psi, dry gas, 11.0 psi, dry temperature, 340 °C, MS/MS, scan from m/z 350 to 1500; ion trap scan from m/z 100 to 1500, maximum accrual time, 100.00 ms; average.

2.5. Nitric oxide radical scavenging activity

The antiradical activity was determined according to Ferreres et al. (2012): 0.5 mL of maize samples (0.1–1.0 mg/mL) were added to 0.5 mL of 25 mM sodium nitroprusside solution and incubated at 25 °C for 1.5 h. At the end of the incubation period, 1 mL of Greiss reagent (prepared from equal volumes of 2% sulfanilamide with 4% H_3PO_4, 0.2% naphthyl thylenediamide) was added to each sample, and the absorbance was read at 562 nm. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. Control samples containing no maize extract or reference were also prepared. Blank samples contained all reagents except Griess reagent to correct for background absorbance conferred by the maize extract or reference. Ferulic acid was used as positive control. Activity was calculated as follows:

$$\text{Percent scavenging activity} = \left\{1 - \left|\left(\text{Absorbance}_{\text{maize of reference}} - \text{Absorbance}_{\text{blank}}\right) \Big/ \text{Absorbance}_{\text{control}}\right|\right\} \times 100$$

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