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Bioactive compounds and protective effect of red and black rice brans extracts in human neuron-like cells (SH-SY5Y)

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ABSTRACT

Rice bran is obtained from the rice polishing process, and this by-product contains many bioactive compounds. In this study, the composition of phenolic compounds from red and black rice brans was determined by HPLC – DAD – MS. Additionally, the neuroprotective ability of these brans in SH-SY5Y cells insulted with hydrogen peroxide (H_2O_2) was evaluated. The phenolic constituents of rice bran were separated into hydrophilic and pellet fractions. The major phenolic compound in both samples was ferulic acid. Cyanidin 3-glucoside was the main anthocyanin in black rice bran. The hydrophilic and pellet fractions showed a protective effect (38–94%) on SH-SY5Y cells insulted by H_2O_2 in DCFH-DA assay. No extract showed cytotoxicity in the SRB assay. These results suggest a neuroprotective effect of red and black rice brans extracts due to their high antioxidant capacity, along with the absence of cytotoxicity. Thus, they may potentially be used as sources of bioactive compounds.

1. Introduction

Pigmented rice grains, such as red and black rice, have the highest content of phenolic compounds distributed in their bran layers (Paiva et al., 2014), mainly *p*-coumaric, ferulic, and protocatechuic acids (Min, McClung, & Chen, 2014). While in black rice the main phenolic constituents are anthocyanins, especially cyanidin 3-glycoside and peonidin 3-glycoside (Min, Gu, McClung, Bergman, & Chen, 2012) in red rice, proanthocyanidins or condensed tannins are the most prominent compounds (Gunaratne et al., 2013).

Because of its high nutrient contents, including fiber, minerals, and phytochemicals, these types of rice have received increasing attention and popularity (Slavin, Martini, Jacobs, & Marquart, 1999). A recent report showed that the ingestion of high cholesterol diet supplemented with red rice promoted hypocholesterolemic effect and increased the concentration of high-density lipoprotein (HDL) in mice (Park, Park, Kim, & Chung, 2014). Furthermore, supplementation with extracts of black rice reduced hepatic steatosis in mice fed a high fat diet (Jang et al., 2012).

Despite these benefits, the consumption of polished rice is still prevalent. The rice polishing process involves a reduction in the nutritional value of the grain as it removes the bran, which is the richest part of the grain in proteins, fibers, vitamins, fat and bioactive compounds. Furthermore, it triggers an economic problem for the processing mills and the rice industry, since it generates a considerable amount of bran (around 8 g/100 g of product).

Thus, the beneficial effects generated in the organism related to the action of the compounds present mainly in the outer layer of red and black rice grains have been reported in some studies using *in vitro* and *in vivo* models. For example, the black rice bran (BRB) fraction prevented DNA damage induced by reactive oxygen species (ROS), namely peroxyl (ROO·) and hydroxyl radicals (HO·) *in vitro* (Hu, Zawistowski,

Abbreviations: ABTS, 2,2 AZINO BIS (3-ethylbenzo thiazoline 6 sulfonic acid); BRB, black rice bran; DCFH-DA, (2′,7′-Dichlorodihydrofluorescein diacetate); DMSO, (dimethyl sulfoxide); DPPH, 2,2-diphenyl-1-picrylhydrazy; HDL, high-density lipoprotein; HO·, hydroxyl; HPLC-MS-DAD, high-performance liquid chromatography coupled to a diode array detector and a mass spectrometer; LOD, limits of detection; LOQ, limits of quantification; ROS, reactive oxygen species; ROO·, peroxyl; RRB, red rice bran; SRB, sulforhodamine B; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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Ling, & Kitts, 2003). Moreover, this same fraction has been reported to reduce oxidative stress and inflammation in apoE-deficient mice (Xia, Ling, Ma, Kitts, & Zawistowski, 2003). Another research has shown that the red rice bran (RRB) extract exhibited inhibitory effects on several cancer cells (Chen, Choi, Kozukue, Kim, & Friedman, 2012) and supplementation of BRB decreased the risk of cardiovascular diseases in humans (Wang et al., 2007). These effects are possibly associated with the high concentration and antioxidant activity of phenolic compounds found in these types of rice.

Oxidative stress underlies the pathogenesis of various neurodegenerative diseases, particularly the damage mediated by ROS, such as hydrogen peroxide (H_2O_2) (Melo et al., 2011). Since neuronal cells exhibit high metabolic activity, non-replicative nature, and low antioxidant capacity, they are very susceptible to ROS actions (Lee, Giordano, & Zhang, 2012). Thus, the development of studies using neuron-like cells, such as the SH-SY5Y cell line, allows the evaluation of the antioxidant activity of compounds in human cells with biochemical characteristics similar to neurons (Ruffels, Griffin, & Dickenson, 2004). In fact, the extract of germinated brown rice was able to prevent H_2O_2-induced oxidative damage in SH-SY5Y cells (Azmi, Ismail, Imam, & Ismail, 2013). Despite the existing reports on the antioxidant activity of red and black rice, the methodology employed is based on scavenging DPPH radical and ABTS radical (cell-free assays) (Paiva et al., 2014). Furthermore, until the present moment, none study is available about the phenolic composition of different fractions from the RRB and BRB and their relationship with antioxidant capacity using a neuron-like cellular system.

Therefore, the goal of this study is to determine the composition of phenolic compounds along with the evaluation of the neuroprotective effects of pigmented rice brans extracts in SH-SY5Y cells.

2. Material and methods

2.1. Chemicals and reagents

Standards of *p*-coumaric acid, ferulic acid, gallic acid, protocatechuic acid, vanillic acid, syringic acid, *p*-hydroxybenzoic acid, caffeic acid, and catechin were purchased from Sigma-Aldrich (St. Louis, MO). All standards had at least 95% purity, as determined by HPLC-DAD. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and ferulic acid were obtained from Sigma-Aldrich (St. Louis, USA). Acetonitrile and methanol (HPLC-grade solvents) were purchased from J.T. Baker (Phillipsburg, NJ). Formic acid was acquired from Merck (Darmstadt, Germany) and acetone, diethyl ether, and sodium hydroxide were purchased from Dinâmica Ltda (Brazil). Ultrapure water was obtained with a Milli-Q system (Billerica, MA). The samples and solvents were filtered through membranes of 0.22 and 0.45 μm pore size, respectively. DMSO (dimethyl sulfoxide), DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate) and SRB (sulforhodamine B) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and H_2O_2 (hydrogen peroxide) was purchased from Vetec (Duque de Caxias, RJ, Brazil).

2.2. Samples

The RRB used in this study was obtained from the processing of red rice (*Oryza glaberrima*) planted in organic soil from southern Brazil (Camaquã, Rio Grande do Sul, Brazil; 30° 51′ 3″ S, 51° 48′ 43″ W) and harvested in April 2013. The BRB was obtained from the processing of organic black rice (*Oryza sativa* – cultivar IAC 600) planted in the city of Sentinela do Sul (Rio Grande do Sul, Brazil; 30° 36′ 39″ S, 51° 34′ 44″ W) and harvested in April 2014. Rice samples (100 g/batch) were polished at Instituto Rio Grandense do Arroz (IRGA) in a mini mill (Suzuki, Japan) for 1.5 min to obtain approximately 10% (w/w) of fresh rice bran. The brans were stored in plastic bags sealed under vacuum at −18 °C until use.

2.3. Extraction of phenolic compounds

Free phenolic acid and insoluble-bound phenolic acid from RRB and BRB were extracted using the procedure described by Naczk and Shahidi (1989) with modifications regarding the amount of sample used and the time during which they were subjected to nitrogen (N_2) flow. The sample was weighed (0.5 g) in a test tube followed by the addition of a mixture of acetone/methanol/water (7:7:6, v/v/v) (40 mL). This mixture was homogenized with a Turrax homogenizer (Quimis, Germany) for 4 min, centrifuged at 4.000 × *g* for 5 min (Hitachi CR 21G III centrifuge, Japan) and the supernatant was collected. This procedure was repeated six times, and the supernatants were combined. The extract was concentrated in a rotary evaporator (T ≤ 30 °C) to approximately 60 mL, transferred to a separator funnel and six extractions were performed with diethyl ether at 1:1 (v/v). From this washing the hydrophilic fraction was obtained which contains free phenolic acids. In parallel, the pellet fraction, characterized by the solid residue, which is resulting from the extractions was subjected to hydrolysis with 20 mL of 4 M NaOH under N_2 flow for 2 h at room temperature (21 °C). The hydrolysate was acidified to pH 2 using 6 M HCl, centrifuged at 4.000 × *g* for 5 min, and the supernatant was transferred to a separator funnel and subjected to six extractions with diethyl ether at 1:1 (v/v). The combined extracts were evaporated in a rotary evaporator (T ≤ 30 °C) in order to obtain the insoluble-bound phenolic acids. The both extracts were stored at freezing temperature (−18 °C) until analysis.

2.4. HPLC-DAD-MS^n analysis

HPLC (Shimadzu, Kyoto, Japan) connected in series to a diode array detector (DAD) (Shimadzu) and a mass spectrometer with a quadrupole-time-of-flight analyzer (Q-TOF MS) and electrospray ionization (ESI) source (Bruker Daltonics, MicroTOF-QIII, Bremen, Germany) was used to characterize the phenolic compounds.

The phenolic compounds were separated on a C_{18} column (5 μm, 250 × 4.6 mm, Atlantis) at a flow rate of 0.7 mL/min and column temperature of 29 °C using a mobile phase consisting of water/formic acid (99.5:0.5, v/v) (solvent A) and acetonitrile/formic acid (99.5:0.5, v/v) (solvent B) in a linear gradient from A/B 99:1 (v/v) to 50:50 (v/v) in 50 min; then from 50:50 (v/v) to 1:99 (v/v) in 5 min. The latter ratio (1:99, v/v) was maintained for a further 5 min (Rodrigues, Mariutti, & Mercadante, 2013). The column eluate was split to allow only around 0.35 mL/min to enter the ESI interface. The UV–vis spectra were obtained between 200 and 600 nm, and the chromatograms were processed at 280, 320, and 360 nm. The mass spectra were acquired with a scan range from 100 to 800 *m/z* (Rodrigues et al., 2013). The phenolic compounds were quantified by HPLC-DAD using eight-point analytical curves of ferulic acid and *p*-coumaric acid (0.5 to 12 μg/mL). The analytical curves were linear (r^2 = 0.9923 and r^2 = 0.9919), the limits of detection (LOD) were 1.45 μg/mL and 1.38 μg/mL, and the limits of quantification (LOQ) were 4.09 μg/mL and 4.19 μg/mL for ferulic acid and *p*-coumaric acid, respectively. LOD and LOQ were calculated according to the parameters of analytical curves (Ribani, Collins, & Bottoli, 2007). Anthocyanins were quantified by HPLC-DAD using a six-point analytical curve of cyanidin 3-glucoside (3.76 to 37.64 μg/mL). The analytical curve was linear (r^2 = 0.9987), LOD was 1.77 μg/mL, and LOQ was 5.38 μg/mL.

Phenolic compounds were identified considering the results of the following parameters obtained from the HPLC-DAD-MS^n system: elution order in the C_{18} column, UV–vis spectral features (maximal absorption wavelength (λ_{max}) and spectrum profile), co-elution with standard, mass spectrum characteristics compared to characteristics of the standard analyzed under the same conditions, and data available in the literature (Qiu, Liu, & Beta, 2010); Shao, Xu, Sun, Bao, & Beta, 2014a).

The MS parameters for both analyses were set as follows: ESI source