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Phenolic acids and flavonoids of peanut by-products: Antioxidant capacity and antimicrobial effects

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ABSTRACT

Peanut skin (PS) and meal from dry-blanched peanuts (MDBP) were evaluated as sources of phenolic compounds. PS rendered the highest total phenolic content, antioxidant capacity towards ABTS radical cation, DPPH and hydroxyl radicals as well as reducing power. Phenolic acids were present in PS and MDBP whereas proanthocyanidins and monomeric flavonoids were found only in PS as identified by HPLC-DAD-ESI-MS^n. Procyanidin-rich extracts prevented oxidation in non-irradiated and gamma-irradiated fish model system. Both extracts inhibited the growth of gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Geobacillus stearothermophilus*) and gram-negative bacteria (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Escherichia coli*). Regardless of the strain, phenolic acid-rich extracts showed the lowest minimum inhibitory capacity (MIC); therefore presenting higher antibacterial effect. The MIC of phenolic acid-rich extracts (24–49 μg phenolics/mL) was higher but comparable to Ampicillin (10 μg/mL). Thus, phenolics in PS and MDBP may serve as antioxidants and antimicrobial compounds.

1. Introduction

Nuts and oilseeds, including peanuts, their products and by-products are well recognized sources of phenolic compounds (Alasalvar & Bolling, 2015). Phenolics and/or polyphenolics may render a wide range of health benefits through prevention of cardiovascular diseases, diabetes, and obesity. Furthermore, their anticancer, *anti*-inflammatory, and antimicrobial effects have also been documented (Lin et al., 2016; Shahidi & Ambigaipalan, 2015). The antioxidant activity of phenolic compounds, which stems from their ability in scavenging radicals by single electron transfer (SET) and hydrogen atom transfer (HAT), is widely studied (Leopoldini, Marino, Russo, & Toscano, 2004). Although phenolic compounds from different resources have been reported to act as scavengers of radicals and other reactive oxygen species (ROS), the deactivation of metal ions due to reduction and/or chelation has also been contemplated (Zhang & Tsao, 2016).

The literature provides extensive data on the antioxidant activity of different phenolic compounds such as phenolic acids, and flavonoids, including anthocyanins (Zhang & Tsao, 2016). Among flavonoid-related compounds, proanthocyanidins (PAC) have received special attention due to their complex structure, which makes their characterization and quantification challenging (Ma et al., 2014; Oldoni et al., 2016). Based on their linkages, PAC are classified as PAC type A and PAC type B. Grapes are rich sources of B-type PAC whereas peanuts are sources of A type PAC (Ma et al., 2014; Melo, Arrivetti, de Alencar, & Skibsted, 2016). The antioxidant activity of PAC from grapes and their products and/or by-products have been well documented whereas PAC from peanuts have received less attention. Peanuts have been used for oil extraction for many years; however, a quick search of the literature reveals little data on the contents of phenolic compounds in peanut meal. Peanut skins (PS) have been used for the development of new food products (de Camargo, Vidal, Canniatti-Brazaca, & Shahidi, 2014; Ma, Kerr, Swanson, Hargrove, & Pegg, 2014) with enhanced content of bioactive compounds. Nevertheless, the use of phenolics from peanut by-products as antioxidants in food

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model systems and as antimicrobial compounds should also be considered.

Gamma irradiation is an effective treatment to reduce and/or eliminate microorganisms in different food products such as spices, oilseeds, meat, and fish, therefore improving their safety and shelf-life (Badr, 2012; Ben Fadhel et al., 2016; Di Stefano, Pitonzo, Bartolotta, D'Oca, & Fuochi, 2014; Kirkin, Mitrevski, Gunes, & Marriott, 2014). However, gamma irradiation induced oxidation and consequent sensory quality changes have been an issue of concern. Thus, antioxidants may serve as an alternative to prevent oxidation, thus improving the process by decreasing oxidative reactions and formation of undesirable chemical products. However, there is a concern about the safety of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) (Shahidi & Ambigaipalan, 2015). Therefore, phenolics from natural resources have been receiving increasing attention as clean label alternatives.

Phenolic compounds have also been reported to act as antimicrobials against pathogenic gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* Typhimurium (Caillet, Cote, Sylvain, & Lacroix, 2012). Several research groups have examined the contamination of foods with these bacteria (Giombelli et al., 2015; Maffei, Alvarenga, Sant'Ana, & Franco, 2016). Additionally, a recent study (Polewski, Krueger, Reed, & Leyer, 2016) demonstrated the synergism among probiotic bacteria and an A type PAC rich extract in diminishing the invasiveness of extra-intestinal pathogenic *E. coli* which may decrease the onset of urinary tract infections in women, thereby demonstrating that the antimicrobial effect of phenolics may also be extended to the field of functional foods.

The antioxidant activity of phenolics from PS has been evaluated in lipid model systems using the Rancimat test (de Camargo, Vieira, Regitano-D'Arce, Calori-Domingues, & Canniatti-Brazaca, 2012). However, to the best of our knowledge, neither PS nor the meal from dry-blanched peanuts (MDBP) has been tested as potential sources of phenolic compounds in a gamma-irradiated fish model system. In addition, the complexity of the mechanism of action of phenolics towards microorganisms has demonstrated that different classes of phenolic compounds and bacteria may influence the effectiveness of these natural compounds in inhibiting their growth. Therefore, in the present study, phenolic extracts from PS and the MDBP were screened for their total phenolic content, antioxidant activity and reducing power. In addition, HPLC-DAD-ESI-MS^n was used for the evaluation of the profile of phenolics present. The application of phenolic extracts as antioxidants was tested in a gamma-irradiated fish model system and their antimicrobial effect was investigated using gram-positive and gram-negative bacteria.

2. Material and methods

2.1. Materials

Dry-blanched peanuts and PS obtained as by-product from the process (cv. Runner 886) were kindly donated by a local company (CAP—Agroindustrial, Dumont, São Paulo state, Brazil). According to the suppliers, the dry-blanching process was carried out at 80 ± 10 °C for 2 h.

Folin Ciocalteau's phenol reagent, DPPH, ABTS, mono- and dibasic potassium phosphates, hydrogen peroxide, DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide), ferrous sulphate, potassium ferricyanide, ferric chloride, butylated hydroxyanisole (BHA), Trolox, catechin and epicatechin as well as protocatechuic, *p*-coumaric, and ferulic acids were purchased from Sigma-Aldrich Canada Ltd. (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). Sodium carbonate, sodium chloride, potassium persulphate, trichloroacetic acid, hexane, acetone, methanol, acetonitrile, formic acid, and hydrochloric acid were purchased from Fisher Scientific Ltd. (Fisher Scientific Ltd., Ottawa, ON, Canada). Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Merck (Merck, Darmstadt, Germany).

Gram-positive bacteria, namely, *Bacillus cereus* (IAL 55), *S. aureus* (ATCC 13565), *Geobacillus stearothermophilus* (ATCC 7953), *L. monocytogenes* (ATCC 7644) and, gram-negative bacteria, namely, *Pseudomonas fluorescens* (ATCC 13525), *Pseudomonas aeruginosas* (IAL 1853), *E. coli* (IAL 2064), *Salmonella* Enteritidis (S 2887), and *S.* Typhimurium (IAL 2431) were used in this study. All strains used in this work were provided by ATCC, IAL (Adolfo Lutz Culture Collection, São Paulo, Brazil) or Institute of Biology (Unicamp, Campinas, Brazil). All culture media used were from Kasvi (Kasvi, Roseto degli Abruzzi, Italy), unless otherwise stated.

2.2. Extraction and characterization of phenolic compounds

2.2.1. Preparation of phenolic extracts

PS and dry-blanched peanuts (50 g) were ground using a coffee bean grinder, Model CBG5 series (Black & Decker, Canada Inc. Brockville, ON, Canada) and the powder was passed through a mesh 16, sieve opening 1 mm, Tyler test sieve (WS Tyler, Mentor, OH, USA). The powder so obtained was defatted three times with hexane (solid/solvent, 1:5, w/v) using a Warring blender, Model 33BL73 (Warring Products Division Dynamics Co. of America, New Hartford, CT, USA) and stored at −20 °C. The sample yield (dry matter) was 53 and 89%, for meal from dry-blanched peanuts (MDBP) and PS, respectively. Defatted samples (2.5 g) were suspended in 70% (v/v) acetone (100 mL) and stirred for 20 min 30 °C in a gyratory water bath shaker, Model. G76 (New Brunswick Scientific Co. Inc., New Brunswick, NJ, USA). After centrifugation at 4000*g* using an IEC Centra MP4 centrifuge (International Equipment Co., Needham Heights, MA, USA), the upper layer was collected and extraction was repeated twice. The combined supernatants were evaporated to remove the organic solvent (de Camargo et al., 2014). The extract so obtained (soluble phenolics) was stored at −20 °C until used for further analysis within three months.

2.2.2. Total phenolic content (TPC)

The TPC was evaluated according to the method of Swain and Hillis (1959) as described elsewhere (de Camargo, Vieira, Regitano-D'Arce, Calori-Domingues, & Canniatti-Brazaca, 2012). The results were reported as milligram catechin equivalents (CE) per gram of defatted sample.

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

HPLC-DAD-ESI-MS^n analysis for positive or tentative identification and quantification of major phenolics were carried out using an Agilent 1100 system equipped with a G1311A quaternary pump, a G1379A degasser and a G1329A ALS automatic sampler, a G1130B ALS Therm, a G1316 Colcom column compartment, A G1315B diode array detector (DAD) and a system controller linked to Chem Station Data handling system (Agilent Technologies, Palo Alto, CA, USA). Separations were conducted with a SUPERLCOSILTM LC-18 column, 4.6 × 250 mm × 5 μm (Merck, Darmstadt, Germany). HPLC-ESI-MS^n analyses were carried out using an Agilent 1100 series capillary liquid chromatography/mass selective detector (LC/MSD) ion trap system in electrospray ionization (ESI) in the negative mode. The data were acquired and analyzed with an Agilent LC/MSD software. Details of the method have appeared elsewhere (de Camargo et al., 2014). Limits of detection and