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Concentrations of oligomers and polymers of proanthocyanidins in red and purple rice bran and their relationships to total phenolics, flavonoids, antioxidant capacity and whole grain color ☆

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ABSTRACT

Proanthocyanidins, a flavonoids subgroup, are proposed to have chronic disease modulation properties. With the eventual goal of enhancing rice phytonutrient concentrations, we investigated the genotypic variation of the concentrations of individual oligomers and polymers of proanthocyanidins in red and purple rice brans. A 4.3-fold variation in total proanthocyanidins (sum of oligomers and polymers) in the extractable fraction was found and the concentration was highly correlated with total phenolics, total flavonoids and antiradical capacity. Variation in the proportion of oligomers and polymers existed, with monomers to trimers, 4–6mers, 7–10mers and polymers accounting for 7, 18, 26.5 and 48.7%, respectively, of the total. The redness value a^* of whole grain rice measured in CIE $L^*a^*b^*$ color space was negatively and positively correlated with extractable and non-extractable proanthocyanidins, respectively. The variation found indicates it is possible to select rice with bran containing high levels of total proanthocyanidins and specific degree of polymerization profiles.

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1. Introduction

Proanthocyanidins (PAs), also known as condensed tannins, are a major subgroup of flavonoids that are oligomers and polymers of flavan-3-ol units. The molecular weights (or degree of polymerization, DP) of these compounds depends on the number of monomeric units (i.e. (+)-catechin and/or (−)-epicatchin) that are linked via an interflavan bond of C4 → C6 or C4 → C8 (B-type) or doubly linked by a C4 → C8 bond and C2 → O7 ether bond (A-type) (Gu et al., 2002). PAs are capable of modulating inflammatory responses, which are involved in the development of cardiovascular disease and some cancers, as well as providing protective effects against type 2 diabetes. Studies reporting these observations have been performed using *in vitro* and *in vivo* methods, with the latter being rather limited in number and scope (Gonzalez-Abuin et al., 2015; Holt, Heiss, Kelm, & Keen, 2012; Martinez-Micaelo, González-Abuín, Ardèvol, Pinent, & Blay, 2012; Ouédraogo et al., 2011). *In-vivo* studies have also indicated that intestinal infections and inflammatory intestinal diseases may be modulated by the consumption of proanthocyanidin (PA) (Monagas et al., 2010).

The DP of PAs influences whether and where these compounds are absorbed in the intestine and thus which metabolites will reach systemic circulation (Monagas et al., 2010; Ou & Gu, 2014). Monomers to trimers are permeable across human intestinal epithelial caco-2 monolayers via paracellular transport (Deprez, Mila, Huneau, Tome, & Scalbert, 2001); however, dimers and trimers have lower absorption rates than monomers (Serra et al., 2010). After reaching the colon, a portion of the non-absorbed PAs is metabolized by the microbiota and the resulting metabolites are absorbed by the colonocytes. These are metabolized by phase II enzymes before entering the systemic circulation, while the remaining PAs are eliminated in the feces (Ou & Gu, 2014). The rate of catabolism of PAs by microbiota in the colon decreases as the DP of the PAs increases (Ou & Gu, 2014). Thus, the greater the molecular weight of PAs the lower their rate of intestinal absorption and microbial catabolism. This generalization, however, does not consider factors that may influence the bioaccessibility of PAs, such as rate of release from the food matrix of a meal.

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Upon intestinal absorption, monomeric flavan-3-ols undergo metabolism by phase II enzymes to form glucuronidated, sulfated or methylated conjugates of flavan-3-ols, whereas the dimers mostly remain intact in circulation (Monagas et al., 2010); while the major microbial metabolites of PAs identified in blood and urine are phenylvalerolactone and other phenolic acids (Monagas et al., 2010; Ou & Gu, 2014). Studies have suggested that PAs might have prebiotic-potential by modulating the gut microbiota and providing health benefit without being absorbed (Ou & Gu, 2014). A human intervention study by Tzounis et al. (2011) demonstrated prebiotic benefits associated with the consumption of a flavanol-rich cocoa drink that significantly increased the commensal bacterial population, *Bifidobacteria* and *Lactobacilli*, and decreased the pathogenic bacteria, *Clostridia*, while concomitantly reducing concentrations of triacylglycerol and C-reactive protein in plasma, biomarkers for cardiovascular risk and inflammation.

Limited information is available regarding the relationship of DP of PAs to their bioactivities. Studies have suggested that size-bioactivity relationships of PA seem to be system dependent. The high molecular-weight PA polymeric fraction from cocoa was more effective at preserving membrane integrity and modulating inflammation than either the monomeric or oligomeric fraction did in an *in vitro* colon cell model (Bitzer et al., 2015); while a long-term mice high-fat feeding study showed that the oligomeric cocoa PA was more effective in preventing diet-induced obesity and insulin resistance than monomeric and polymeric fractions (Dorenkott et al., 2014). The oligomers from a PA extract from grape seed were more effective than polymers in improving serum lipid profiles of diabetic rats (Wu et al., 2015). More *in vivo* studies need to be conducted to clarify the bioactivities of PA, including evaluations of the absorption rate and metabolism of these compounds.

Based on the current knowledge of the relationship of the DP of PAs with absorption, metabolism, and bioactivity, both the total quantity and the DP distribution of PAs (DP proportion) must be considered when studying PA-containing foods (Monagas et al., 2010). Gu et al. (2004) screened common foods and found that the total concentrations and the DP distributions of PAs vary widely among different foods and within each type of food (USDA database for the proanthocyanidin content of selected foods, 2004, http://www.ars.usda.gov/News/docs.htm?docid=5843).

In rice, phytochemicals are concentrated in the bran layer (bran and germ), the outer layer of whole grain rice that gives the color of the whole grain (Shao & Bao, 2015; Shao, Xu, Sun, Bao, & Beta, 2014). Most whole grain rice (with the bran layer intact) sold commercially is light brown in color and does not contain PA (Pereira-Caro, Cros, Yokota, & Crozier, 2013); while red and some purple rice varieties contain PAs and have several fold higher antiradical capacity than light brown bran rice (Finocchiaro, Ferrari, & Gianinetti, 2010; Min, Gu, McClung, Bergman, & Chen, 2012). PA-rich fractions extracted from red rice bran reportedly suppress the growth of various cancer cells *in vitro* (Chen, Choi, Kozukue, Kim, & Friedman, 2012). Thus, rice with high PA has the potential to be sold as specialty rice or as its PA extract as a dietary supplement. Shen, Jin, Xiao, Lu, and Bao (2009) reported wide ranges of total phenolic concentration and antioxidant capacity of rice with red bran; Shao et al. (2015) determined the total PAs in 16 red bran rice cultivars using a colorimetric method and a 2.7-fold variation was found. Thus far, little information is available regarding the genotypic diversity in DP distribution of PAs in rice.

It is often the case that rice germplasm with special quality traits are landraces or cultivars that are not well adapted to today's cultural management practices nor have the yield potential of conventional cultivars. Development of rapid screening methods for selecting for PA concentration in rice germplasm would facilitate a breeding effort for enhancing this trait, while maintaining high yield potential and disease resistance (McClung, 2004). Therefore, this study has two main objectives. Firstly, evaluate the genotypic variation in red and purple bran of rice of diverse geographic origins for concentrations of oligomers and polymers of PAs, and their relationships to the total phenolic and flavonoid concentrations and antiradical capacity. Secondly, evaluate the suitability of a non-destructive grain color method and a high throughput colorimetric procedure for selecting for PA concentration of red and purple rice or bran.

2. Materials and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), gallic acid, sodium nitrite, aluminum chloride, ammonium ferrous sulfate, fluorescein disodium, procyanidin B2, 4-(dimethylamino) cinnamaldehyde (DMAC) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO., U.S.A.). (+)-catechin, and Folin-Ciocalteu reagent were obtained from Fluka (Milwaukee, WI). The 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA., U.S.A.). Cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-O-rutinoside, and cyanidin chloride were purchased from Sigma-Aldrich (St. Louis, MO., U.S.A.), peonidin-3-glucoside from Extrasynthèse (Genay, France), and peonidin chloride, delphinidin-3-glucoside, and delphinidin chloride from Chromadex (Irvine, CA., U.S.A.). Methanol and acetone were HPLC grade from Fisher Scientific (Fair Lawn, NJ., U.S.A.). All other chemicals and reagents used were of reagent grade.

2.2. Rice accessions and sample preparation

Thirty-two rice accessions (see Table 1 in Chen, McClung, & Bergman, 2016) were obtained from the USDA National Small Grains Collection (NSGC). Five accessions were originated from the continent of African, 17 from Asia, 1 from Europe, 4 from North American, 2 from South American, and 3 were uncertain. Detailed information for each accession can be accessed through GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?75011). Four accessions had purple bran and 28 accessions had red color bran. Our classification of bran color was based on visual observation. Three accessions were classified as light brown or brown color in GRIN; however, the bran color turned red upon storage, thus we classified these three accessions as red bran (see Table 1 in Chen et al., 2016). In the literature, most references to rice with purple-colored bran describe the color as black, however we classify them as purple to follow the color classification described by the NSGC. During 2009 and 2010 the accessions were grown in Beaumont, Texas (U.S.A.) using unreplicated flooded field plots that were approximately 0.54 m^2. Plots were drill seeded on May 5, 2009 and on April 20, 2010 using a seeding rate of 110 kg ha^{-1}. Pest management practices were standard for the growing area. A relatively low rate of urea fertilizer (73 kg ha^{-1}) was applied in a 2-way split to limit lodging. Rough rice samples were harvested at approximately 20% moisture, dried to 12% moisture, and dehulled using a rice huller (Satake, Tokyo, Japan; model THO35A). The whole grain (i.e., dehulled) kernels were milled using a McGill mill #1 (HT McGill, Houston, TX) (Chen & Bergman, 2005). The bran (the word bran is used to mean bran and germ collectively) was collected, sieved through a 28-mesh screen, flushed with nitrogen, and stored at −20 °C until further analysis.

2.3. Sample extraction

The following extraction procedure was used to produce sample extracts for the analyses of extractable total phenolic, total