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Structural profile of soluble and bound phenolic compounds in teff (*Eragrostis tef*) reveals abundance of distinctly different flavones in white and brown varieties

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ABSTRACT

Reliable data on polyphenol in teff, an increasingly important food crop, is currently lacking. This study investigated the structural and quantitative profile of soluble and bound polyphenols in white and brown teff grown in Ethiopia and USA using LC-tandem quadrupole mass spectrometry. Thirty-four phenolic compounds (32 newly identified in teff), mostly flavones and phenolic acid derivatives, were characterized. Unusually high levels of flavones were present in both white (1398–2049 μg/g), and brown (1720–1847 μg/g) teff soluble fractions. Interestingly, white teff exclusively contained apigenin derivatives, whereas brown teff contained mainly luteolin derivatives, mostly di-C-linked-glycosides. Additionally, non-extractable procyanidins (condensed tannins) were detected in brown teff only. Phenolic acids (600–728 μg/g) were mostly present in bound fractions, dominated by ferulic acid. Polyphenol profiles of Ethiopian and USA grown grains were similar. The high levels of the relatively rare flavones in teff may have important implications in chronic disease prevention.

1. Introduction

Grain teff (*Eragrostis tef*) is a tropical cereal, native to Ethiopia, where it is a major staple (Shumoy & Raes, 2016). Generally referred to as tef or teff, it is the smallest cultivated grain in the world, is adapted to a range of environmental growing conditions and is also less susceptible to attack by pests (Gebremariam, Zarnkow, & Becker, 2014). This grain is gaining popularity because it is gluten free and very rich in nutrients, especially minerals such as calcium, iron, and magnesium, compared to other grains such as wheat, barley, sorghum and pearl millet (Gebremariam et al., 2014). Demand for teff is growing, and it is currently produced in countries such as United States, South Africa, Australia and Canada (Taylor, 2017, chap. 4).

Epidemiological evidence has shown that whole grain consumption is strongly associated with reduced risk of chronic diseases such as cancer, diabetes, cardiovascular disease (Aune et al., 2016; Wu et al., 2015). Among the whole grain components that contribute to the benefits are dietary fiber and associated polyphenols. Polyphenols are especially important components of whole grains, because their composition and content have major impact on sensory properties (color, flavor, etc) of whole grain-based products and consumer choices of such products (Awika & Duodu, 2017). For example, in Ethiopia, white teff is preferred over brown teff by most consumers (Belay et al., 2006; Gebremariam et al., 2014).

On the other hand, whole grain polyphenols have been shown to directly influence various pathways relevant to chronic disease prevention in important ways, with structural profile of the polyphenols being a major predictor of their biological function (Guo, Wise, Collins, & Meydani, 2008; Vitaglione et al., 2015; Yang, Allred, Dykes, Allred, & Awika, 2015; Yang, Allred, Geera, Allred, & Awika, 2012). Furthermore, some of the polyphenols, for example, the polymeric proanthocyanidins (condensed tannins), can bind proteins and carbohydrates, significantly reducing their digestibility (Amoako & Awika, 2016; Taylor, Bean, Ioerger, & Taylor, 2007) and thus impacting nutritional quality of food. Therefore, the composition of polyphenols in cereal grains is important, not only from a consumer sensory perspective, but also in helping predict their potential effect on nutrient bioavailability, and chronic disease prevention.

There is surprisingly little that is known about the phenolic profile of teff; and as yet, the compounds responsible for the intense pigmentation in the brown teff pericarp are unknown. The few studies available on teff (El-Alfy, Ezzat, & Sleem, 2012; Habtu & Katleen, 2016; Kotásková, Sumczynski, Mlček, & Valášek, 2016; Salawu, Bester, & Duodu, 2014) are very limited in scope and provide little structural

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information and/or reliable quantitative data on teff phenolics. Not surprisingly, there is little agreement on the type of compounds identified across the studies. Thus, the phenolic composition of teff remains largely unknown. In this study, we use UPLC-tandem quadrupole MS/MS to profile the structure and content of soluble (free) and bound polyphenols in white and brown teff grains grown in Ethiopia and USA.

2. Materials and methods

2.1. Plant materials

White and brown teff grains grown in Idaho, USA (Shiloh farms, PA, USA) in 2016 were commercially purchased. In addition, white and brown teff were purchased from a local market in Hawassa, Ethiopia in 2017. The whole seeds were ground using UDY mill to pass through a 1 mm mesh sieve and stored at −20 °C until used.

2.2. Chemicals and reagents

All reagents were analytical grade. Apigenin, apigenin-7-O-glucoside, luteolin, luteolin-7-O-glucoside and cyanidin chloride were purchased from Extrasynthese (Genay Cedex, France), ferulic acid was from Indofine (Hillsborough, NJ, USA) and p-coumaric acid was from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Extraction of soluble and bound polyphenols

Extraction of soluble phenolics was performed according to Liu, Qiu, & Beta (2010) and Shumoy & Raes (2016), with slight modifications. Ground teff was extracted in 80% methanol (1:5 flour:solvent, w/v) for 2 h with shaking at 200 cycles/min at room temperature (Standard Analog Shaker, VWR, Radnor, PA). The extracts were centrifuged (10,000*g* for 10 min) using a Heraeus Megafuge 11R Centrifuge (Thermo Fisher Scientific, Asheville, NC) at 20 °C and the supernatant was transferred to new set of tubes. The residue was further extracted twice (1:2.5 residue:solvent (80% methanol), w/v) each time for 30 min and centrifuged. The supernatants were combined and stored at −80 °C until further use.

Extraction of bound phenolics was performed according to Liu et al., (2010) and Shumoy, Gabaza, Vandevelde, & Raes, (2017) with slight modifications. Briefly, 1 g of dried residue (from the soluble phenolics extraction) was hydrolyzed in 30 mL of 2 M NaOH for 30 min at 60 °C. The samples were then centrifuged at 10,000*g* for 30 min and the supernatant was decanted into new set of tubes. The pH of the supernatant was adjusted to 1.5–3 by using 6 N HCl and extracted once with 30 mL hexane to remove lipids. The phenolics were then extracted twice with ethyl acetate (once with 30 mL and 2nd time with 20 mL). The ethyl acetate fractions were combined and dried using rotary evaporator under vacuum (Buchmann R110 Rotavapor, Westbury, NY). The residue was dissolved in 1 mL 80% methanol and stored at -80 °C until further use.

2.4. Acid catalyzed thermal hydrolysis to test for presence of proanthocyanidins

Based on phenolic profiles obtained from above extraction protocols, we were unable to determine the compounds responsible for the dark reddish-brown pigmentation in the brown teff pericarp. In other grains like sorghum and finger millet, such pigmentation is typically a function of a pigmented testa containing proanthocyanidins. We thus theorized that insoluble condensed tannin-like compounds were likely responsible. To test this, we used the acid catalyzed thermal hydrolysis and oxidation principle (Porter, Hrstich, & Chan, 1985) to depolymerize and oxidize any present proanthocyanidins to their respective anthocyanidins. Dried residue (from the soluble phenolics extraction) was hydrolyzed using 1% HCl in methanol (1:5 residue:solvent, w/v) using a Microwave Accelerated Reaction System (MARS 5 Xpress, CEM corporation, Matthews, NC). The power was set at 600 W, temperature at 100 °C and reaction was carried out for 10 min. The samples were then centrifuged (10,000*g* for 10 min) using a Heraeus Megafuge 11R Centrifuge (Thermo Fisher Scientific, Asheville, NC) at 20 °C and the supernatant stored at −80 °C until analysis. Purified proanthocyanidins obtained from sorghum was initially used to develop and validate the hydrolysis method.

2.5. Ultra performance liquid chromatography-tandem quadrupole MS/MS analysis

Identification and quantification of polyphenols in teff grains was performed according to Ojwang, Dykes, & Awika (2012) with slight modification on a Waters-ACQUITY-UPLC-TQD-MS/MS system (Waters Corp., Milford, MA) equipped with a photodiode array eλ detector and interfaced with a mass spectrometer equipped with a tandem quadrupole (TQD) electrospray ionization (ESI) detector. The separation was performed on a Kinetex C18 column (100 × 2.10 mm, 2.6 μm) (Phenomenex, Torrance, CA) at 40 °C with flow rate of 0.4 mL/min: The mobile phases consisted of 0.05% formic acid in water (solvent A) and 0.05% formic acid in acetonitrile (solvent B) for phenolic acid and flavone analysis whereas 1% formic acid in water (solvent A) and 1% formic acid in acetonitrile (solvent B) was used for anthocyanin analysis. The percentage of solvent B was 0–5 min 5% B, 5–35 min 35% B, 35–45 min 70% B, 45–50 min 70% B, 50–53 min 5% B and 53–60 min 5% B. For MS/MS analysis, the phenolic extracts were filtered using a syringe filter with a 0.22 μm PTFE membrane and the injection volume was 2 μL, whereas for quantification, the combined supernatants were injected at a volume of 5 μL. Flavones were monitored at 340 nm, other phenolic acids at 280 nm and 325 nm, anthocyanins at 480 nm and 520 nm and mass spectrometric data were acquired in negative mode for phenolic acids and flavones and in positive mode for anthocyanins. The source, ionization gas flow and data processing conditions were similar to that reported by Ojwang et al. (2012). The MS scan was recorded in the range of 100–1000 Da. Parent ion scanning mass parameters were optimized as follows: Capillary voltage was 3.5/3 kV; and cone voltage was set at 30 V for positive/negative ionization respectively. The MS/MS scan was optimized as follows: cone voltage of 30 V and collision energy of 15–40 V. Compound identification was done based on matching UPLC retention profile, UV–vis spectra and MS data with authentic standards. Where standards were not available, compounds were identified based on the fragment patterns compared with reports in literature.

Quantification of the compounds was done by interpolating peak areas based on standard curves of corresponding pure compounds. In the cases where no standards were available, quantification was done based on the assumption that their molar absorptivity was similar to those of their monoglucosides (apigenin-7-O-glucoside for apigenin derivatives, luteolin-7-O-glucoside for luteolin derivatives, ferulic acid for the monomers and ferulic acid dehydrodimers and coumaric acid for the corresponding monomer) similar to the procedure followed by Ojwang et al. (2012). Data was reported on dry basis, based on three separate runs.

2.6. Statistical analysis

Three replications of each treatment were performed. Analysis was done using JMP pro 12 (Version 12.0.1, SAS Institute, Inc., Cary, NC) with one-way Analysis of Variance (ANOVA). Post Hoc test (Fisher's LSD and Tukey-Kramer HSD) was used to compare treatments means. Significance levels were defined using $p < 0.05$.