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A comparative study of anthocyanin distribution in purple and blue corn coproducts from three conventional fractionation processes

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ABSTRACT

The aim was to compare the distribution of ANCs in purple and blue corn coproducts from three conventional corn fractionation processes and linking ANC partitioning in different coproducts to corn kernel phenotype. Total monomeric anthocyanin (TA) from purple corn extract was 4933.1 ± 43.4 mg cyanidin-3-glucoside equivalent per kg dry corn, 10 times more than blue corn. In dry milled purple corn, maximum ANCs were present in the pericarp (45.9% of total ANCs) and in wet-milling they were concentrated in steeping water (79.1% of total ANCs). For blue corn, the highest TA was in small grits and gluten slurry in dry-milling and wet-milling coproducts, respectively. HPLC showed the highest concentration of each ANC in steeping water for purple corn coproducts. Micrographs of kernel showed pigments concentrated in pericarp layer of purple but only in aleurone of blue corn. ANCs can concentrate in certain coproducts depending upon physical distribution of pigments in kernel.

1. Introduction

Anthocyanins (ANCs) are vacuolar pigments belonging to the flavonoid class of plant secondary metabolites and are responsible for vivid colors in a variety of fruits and vegetables. The consumption of ANCs from fruits and vegetables has been associated with various health benefits. Studies have shown that ANCs have antioxidant (Fernandes, Faria, Calhau, de Freitas, & Mateus, 2014; Reque et al., 2014), anti-cancer (Fernandes et al., 2014), anti-obesity (Esposito et al., 2015; Johnson, Wallig, Luna Vital, & de Mejia, 2016), and anti-inflammation effects (Esposito, Chen, Grace, Komarnytsky, & Lila, 2014). However, it is unknown whether these health benefits are solely due to ANCs or the synergistic effect of diverse phytochemicals.

Corn is the most important feed grain in the U.S., accounting for more than 95 percent of total feed grain production and use (USDA, 2016). Out of 13.6 billion bushels of corn utilized in 2015, corn milling industry processed almost 39.2% (National Corn Growers Association, 2016). The dry-grind industry was the largest processor of corn (30.3%), producing fuel ethanol as the primary product and distillers dried grains with solubles (DDGS) as a coproduct. DDGS is mainly used as a feed for ruminants. The wet-milling industry is another major processor (7.4%), producing starch as the primary product and corn gluten meal, corn gluten feed, corn germ, and fiber as the coproducts (National Corn Growers Association, 2016). Corn starch is further processed to produce high fructose corn syrup, modified starches, and sweeteners. The wet-milling industry produces diverse products ranging from antibiotics, adhesives, and amino acids to edible gums (Corn Refiners Association, 2015). The dry-milling industry is the smallest among the three, processing only about 1.5% of the total corn. The primary product from corn dry-milling is large grits. Coproducts include small grits, fines, germ, and pericarp. Large grits are used for making corn flakes and cereal bars while coproducts are utilized in the brewing industry and in making corn flour and hominy feed (National Corn Growers Association, 2016).

Colored corn is one of the richest sources of ANCs, with concentrations ranging from 51 mg cyanidin-3-glucoside equivalent (C3G)/kg in red corn to 1300 mg C3G/kg fresh weight (fw) in purple corn (Abdel-Aal, Young, & Rabalski, 2006). Some studies have shown the amount to be almost 6 g/kg (Yang et al., 2009). Other well-known sources of ANCs include raspberry with about

Abbreviations: ANC, anthocyanin; CF, condensed form; C3G, cyanidin-3-glucoside; GAE, gallic acid equivalent; Pg3G, Pelargonidin-3-glucoside; Pg36MG, Pelargonidin 3-O-(6″-malonyl-glucoside); Pn3G, Peonidin-3-glucoside; C36MG, Cyanidin 3-O-(6″-malonyl-glucoside); Pn36MG, Peonidin 3-O-(6″-malonyl-glucoside); TA, total monomeric anthocyanin; TP, total polyphenol.

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500 mg C3G/kg fw, blackberry with ANCs up to 1500 mg C3G/kg fw (Pantelidis, Vasilakakis, Manganaris, & Diamantidis, 2007), and black rice with ANCs up to 3300 mg/kg (Abdel-Aal et al., 2006). Blue corn is another type of colored corn, although its ANC concentration is not as high as in purple corn. Large scale economical cultivation of corn and its long shelf-life makes it a very attractive source for efficient natural pigment extraction. In addition to variability in total ANC concentration, colored corn contains a number of different anthocyanin species including cyanidin, pelargonidin, and peonidin based monoglucosides, malonyl and dimalonyl glucosides, and flavonol anthocyanin condensed forms.

The objectives of this study were to ascertain and compare the distribution and yield of ANCs from purple and blue corn in various coproducts streams of wet-milling, dry-milling and dry-grind processes to identify the coproducts with maximum concentration of ANCs. Corn coproducts from these three processes methods were analyzed for total ANCs concentration and ANCs profile. Also, the distribution of ANCs in various coproducts depending upon the variability in kernel morphology was considered.

2. Materials and methods

2.1. Materials

Purple corn (*Zea mays L.*) was procured from specialty foods vendor Angelina's Gourmet (Swanson, CT). Jerry Peterson Blue Organic corn was purchased from Johnny's Selected Seeds (Fairfield, ME). All the reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

2.2. Milling processes

One kg lab-scale milling processes were employed in triplicates for colored corn milling. Moisture content of coproducts was determined with an air-oven at 135 °C for 2 h (AACC International, 2010). For wet-milling, an adapted 1 kg lab-scale procedure developed by Eckhoff et al. (1993) was used. For purple corn, wet-milling coproduct yields (dry weight) were starch, germ, fiber, gluten, and steeping water solids 63.4%, 6.2%, 10.9%, 13.8%, and 5.3%, respectively. Fraction yields (starch, germ, fiber, gluten, and steeping water solids) for blue corn were 61.5%, 8.0%, 10.6%, 14.2%, and 5.2%, respectively. For dry milling, 1 kg lab-scale dry-milling procedure developed by Rausch et al. (2009) was used. Fraction yields for purple corn (large grits, small grits, fines, pericarp, and germ) were 21.8%, 22.2%, 36.1%, 9.9%, and 9.4%, respectively. Fraction yields for blue corn (large grits, small grits, fines, pericarp, and germ) were 24.4%, 24.6%, 28.1%, 12.5%, and 9.5%, respectively. For dry-grind, 1 kg lab-scale process used by Khullar, Sall, Rausch, Tumbleson, and Singh (2009) was employed. Final ethanol concentrations for purple and blue corn were 14.5% and 14.3% (v/v) and DDGS yields were 41.6% and 38%, respectively on dry weight. Somavat, Li, de Mejia, Liu, and Singh (2016) found that colored corn can effectively be used as an alternate feedstock for all the three conventional processes with some yield differences; however, relative value of coproducts can offset these differences.

2.3. Microscopy of colored corn

To soften the kernels and facilitate sectioning, purple and blue kernels were soaked in deionized water for 12 h. Kernels were then saggitally sectioned with a razor blade and embedded in 15 mm × 15 mm × 5 mm disposable vinyl specimen molds (Sakura Finetek USA, Inc., Torrance, CA, USA) using Optimal Cutting Temperature (O.C.T.) compound (Thermo Fisher Scientific, Waltham MA, USA). Embedded kernels were frozen to -15 °C and subsequently sectioned at 14 μm using a Leica Reichert Cryocut 1800 cryostat (Leica Biosystems, Buffalo Grove, IL, USA). Bright field images of pericarp and aleurone layers were acquired using a Canon EOS Rebel T3i Digital SLR Camera (Canon U.S.A. Inc., Lake Success, NY, USA) attached to an Olympus BX51 microscope (Olympus America Inc., Lombard, IL, USA) at 40× magnification.

2.4. Extraction of phenolic compounds from corn coproducts

The following coproducts were analyzed 1) dry-milling coproducts: pericarp, large grits, small grits, fines, germ; 2) wet-milling coproducts: steeping water, gluten slurry, gluten, starch, germ and fines; 3) dry-grind coproduct: DDGS. The solid coproducts were ground using a coffee grinder (Kitchen-Aid, Benton Harbor, MI) for 25 s. The ground material was passed through a 35-mesh sieve, and the material that did not pass through was ground again for another 25 s and passed through a 35-mesh sieve. Materials that passed through the sieve were combined and used for extraction. Approximately 0.5 g of ground material was suspended in 20 mL (40:1 liquid-to-solid ratio) 2% aqueous formic acid and stirred for 2 h at room temperature. The suspension was filtered with Grade 1 Whatman® filter paper, and the resulting filtrate was used to determine total monomeric ANCs, total polyphenols, condensed tannins, color, and HPLC/MS-MS analysis. After the first extraction, coproducts were added into 20 mL 2% formic acid again and stirred at room temperature for 2 h for a second extraction. The suspension from the second extraction was also filtered; the filtrate was collected for further measurements. A third sequential extraction was conducted by mixing each one of the coproducts with 20 mL 2% formic acid with 25% ethanol to further extract ANCs. The mixture was stirred at room temperature for 2 h and the filtrate was collected.

2.5. Measurement of monomeric anthocyanin concentrations

The analysis for total monomeric ANCs concentration was performed by the pH differential method as previously reported (Lee, Durst, & Wrolstad, 2005) using a microplate reader method in three independent replicates. Extracts obtained previously or aqueous coproducts from the wet-milling process such as steeping water and gluten slurry were diluted using two buffers (pH 1.0, 0.25 M KCl buffer and pH 4.5, 0.40 M sodium acetate buffer). Two hundred μL of diluted solutions at each pH were transferred to a 96-well plate, and the absorbance was read at 520 and 700 nm using a Synergy 2 multi-well plate reader (Biotek, Winooski, VT). The total monomeric ANC concentration was calculated as mg of cyanidin-3-*O*-glucoside (C3G) equivalents per L as described below:

$$\text{Total monomeric ANCs (mg/L)} = (A * MW * D * 1000)/(\varepsilon * PL^{*}) \times 0.45$$

where: A = (A520–A700) at pH 1.0 – (A520–A700) at pH 4.5; MW = 449.2 g/mol for C3G; D = dilution factor; PL = constant path length 1 cm; ε = 26,900 L/mol·cm which is the molar extinction coefficient for C3G, 1000 as conversion factor from grams to milligrams and 0.45 as the conversion factor from the established method to the plate reader method. Final results were then expressed as mg C3G equivalents per g co-product. Gluten slurry, a wet-milling co-product, was filtered, and the filtrate was used for ANC concentration determination directly.

2.6. Measurement of total polyphenol concentration

Total polyphenols were measured using the Folin-Ciocalteu method adapted to a microassay, (Bower, Real Hernandez,