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Effect of the storage time and temperature on phenolic compounds of sorghum grain and flour

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ABSTRACT

This study evaluated the effect of storage temperature (4, 25 and 40 °C) and time on the color and contents of 3-deoxyanthocyanins, total anthocyanins, total phenols and tannins of sorghum stored for 180 days. Two genotypes SC319 (grain and flour) and TX430 (bran and flour) were analyzed. The SC319 flour showed luteolinidin and apigeninidin contents higher than the grain and the TX430 bran had the levels of all compounds higher than the flour. The storage temperature did not affect most of the analyzed variables. The content of most of the compounds reduced during the first 60 days when they became stable. At day 180, the retention of the compounds in the genotypes SC319 and TX430 ranged from 56.1–77.9% and 67.3–80.1% (3-deoxyanthocyanins), 88.4–93.8% and 84.6–96.8% (total anthocyanins) and 86.7–86.8 and 89.4–100% (phenols) respectively. The retention of tannins ranged from 56.6 to 85.3%. The color of samples remained stable for 120 days.

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a staple food grain in many semi-arid and tropic areas of the world, particularly in Africa, India, and Asia, because of its good adaptation under various environmental stresses and high yield (Awika & Rooney, 2004; Dicko, Gruppen, Traoré, Voragen, & Berkel, 2006).

Some studies indicate the potential benefits of using sorghum for food consumption because of its bioactive compounds, as the phenolics, that include phenolic acids, flavonoids and condensed tannins (Awika & Rooney, 2004). These compounds, due to their high antioxidant capacity, may help reducing the risk of developing chronic noncommunicable diseases, such as diabetes, obesity, hypertension, cardiovascular diseases and cancer (Awika &

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Rooney, 2004; Cardoso et al., 2014; Yang, Browning, & Awika, 2009).

Phenolic compounds are originated from the secondary metabolism of plants, when they are subjected to stress conditions, such as infections, mechanical injuries and radiation (Naczk & Shahidi, 2004). They are known to play a natural defensive role in the plant by protecting against pests and diseases (Jambunathan, Butler, Bandyopadhyay, & Mughogho, 1986). In sorghum, these phenolic compounds are concentrated in the pericarp of the grain (Moraes et al., 2015).

The anthocyanins are important flavonoids that provide color to the fruits and vegetables. The sorghum anthocyanins, named 3-deoxyanthocyanins (3-DXAs), comprise luteolinidin and apigeninidins and their methoxylated derivatives 5-methoxyluteolinidin and 7-methoxyapigeninidin (Dykes & Rooney, 2006). These 3-DXAs are so called for not having the hydroxyl group at C-3 position (Clifford, 2000). This unique feature of the 3-DXAs provides greater stability to pH changes when compared to those commonly found in vegetables and fruits (Devi, Saravanakumar, & Mohandas, 2012; Mazza & Brouillard, 1987).

The sorghum tannins, also known as proanthocyanidins, are high-molecular weight polyphenols (Dykes & Rooney, 2006) that is located in the testa, a structure located between the pericarp and the endosperm of the grain of some varieties (Earp, McDonough, & Rooney, 2004). Tannins are known to bind to proteins, carbohydrates and other nutrients, limiting the nutritional value of food and decreasing its digestibility (Barros, Awika, & Rooney, 2012; Rubanza et al., 2005), although may bring health benefits for special diets aimed at weight loss. Due to this ability to bind to free radicals, sorghum genotypes containing tannins have higher antioxidant capacity than sorghum that does not contain tannins (Awika & Rooney, 2004).

Although sorghum has potential health benefits due to these compounds, it is of great significance to evaluate their stability during storage, because factors such as time and temperature can affect their concentrations. A study on the effects of domestic processing with dry heat and wet heat on the bioactive compounds of sorghum were evaluated by Cardoso et al. (2014) which found that dry heat did not affect the content of 3-DXAS, total phenols and antioxidant capacity, but the same did not occur when subjected to wet heat. However, studies that elucidate the effect of the temperature and the time of storage on the content of bioactive compounds in sorghum were not found.

This work aimed to evaluate the effect of the storage temperature and time on the color and content of total anthocyanins, 3-deoxyanthocyanins, and condensed tannins of the sorghum genotypes SC319 (grain and flour) and TX430 (bran and flour).

2. Materials and methods

2.1. Samples

The sorghum genotypes SC319 (grain and flour) and TX430 (flour and bran) were used in the trial. The genotype SC319, with brown pericarp and pigmented testa (with condensed tannins), was selected among 100 genotypes of a panel with high genetic variability, due to its anthocyanins (3-deoxyanthocyanins) and tannin contents (unpublished data). This genotype was grown in experimental fields of Embrapa Maize and Sorghum, in Sete Lagoas, Minas Gerais, Brazil, in 2013. After harvesting, the grains were threshed and stored at −18 °C until use. Whole sorghum grains were ground twice in a Hawos mill to obtain particles of the 0.5 mm screen before storage.

The sorghum flour and bran of the genotype TX430, with black pericarp and without pigmented testa (no condensed tannins) (Dykes, Rooney, Waniska, & Rooney, 2005) were supplied by CQL-Cereal Quality Lab., from the Texas A&M University, College Station, TX, USA. This sorghum genotype was grown in College Station, TX, in 2013. After harvesting, the grains were milled using a UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO). The bran was obtained by decorticating the sorghum grains in a PRL mini-dehuller (Nutama machine Co., Saskatoon, Canada) and separated with a KICE grain cleaner (Model 6DT4-1, KICE Industries Inc., Wichita, KS).

2.2. Storage of samples

Sorghum genotypes SC 319 (grains and flour) and TX430 (flour and bran) were placed in individual polypropylene packages with 10 g capacity. Subsequently the packages were placed in paper bags to protect from light and stored in three BOD Refrigerated Incubators (SOLAB 200/334) for a period of 180 days, at three temperatures, 4 ± 2, 25 ± 2 and 40 ± 2 °C. The analysis were performed at 0 (zero, T0), 60 (T60), 120 (T120, except for 3-DXAs) and 180 (T180) days of storage. Sorghum grains (from the sorghum genotype SC 319) remained intact during the storage period and were ground before the analytical procedures.

2.3. Reagents and standard curve

The standards of luteolinidin chloride, gallic acid and catechin hydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). The apigeninidin chloride was obtained from Chromadex (Santa Ana, CA, USA). The analytic grade reagents acetone, chloroform, methanol, and hydrochloric acid were purchased from VETEC (São Paulo, Brazil) and vanillin, Folin-Ciocalteu and ethanolamine from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC) grade reagents (acetic acid, acetone, acetonitrile, ethyl acetate, hexane, isopropyl alcohol, methyl alcohol, and formic acid) were purchased from Tedia (São Paulo, Brazil).

2.4. Deoxyanthocyanins (DXAs) analysis

The 3-deoxyanthocyanins: luteolinidin (LUT), apigeninidin (API), 5-methoxyluteolinidin (5-MeO LUT) and 7-methoxyapigeninidin (7-MeO API) contents were determined according to a method proposed by Yang, Allred, Geera, Allred, and Awika (2012), modified by Cardoso et al. (2014). The compounds were extracted from 1 g of sample with 10 mL of 1% HCl in methanol. Analyzes were performed in an HPLC system (Shimadzu, SCL 10AT VP, Japan) equipped with diode array detector (Shimadzu, SPD-M10A, Japan), high pressure pump (Shimadzu, LC-10AT VP, Japan), autosampler with loop of 500 mL (Shimadzu, SIL-10AF, Japan), and helium degassing system using the chromatographic conditions described by Cardoso et al. (2014).

Identification was performed by correlating the retention time and the absorption spectrum of peaks of the standards and samples, analyzed under the same conditions. The quantification of each compound was performed by comparison of peak areas with those of standard curves constructed through injection, in duplicate, of six different standard concentrations (R^2 ranged from 0.9939 to 0.9992). The 5-MeO-LUT and 7-MeO-API contents were quantified using standards of luteolinidin and apigeninidin, respectively, as well as with the appropriate molecular weight correction factor (Dykes, Seitz, Rooney, & Rooney, 2009). Results were expressed in μg/g dry matter.

2.5. Preparation of crude sorghum extracts for total phenols and total anthocyanins analysis

Phenolic extracts were obtained from sorghum flours (from both genotypes) and bran (from TX430). 0.25 g of sorghum