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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

samples was mixed with 25 mL of 1% HCl/methanol (v/v) and stirred for 2 h. Then, samples were filtered through qualitative filter paper and the extracts were immediately analyzed.

2.6. Total phenols analysis

The modified Folin-Ciocalteu method of Kaluza, McGrath, Roberts, and Schröder (1980) was used. One aliquot (0.1 mL) of acidified methanol extract was mixed with 1.1 mL of distilled water then reacted with 0.4 mL Folin-Ciocalteu reagent and 0.9 mL 0.5 M ethanolamine for 20 min at room temperature. The absorbance was read on a spectrophotometer (Hitachi UV–Visible 1100) at 600 nm. A five points standard curve dilutions of gallic acid (0, 5, 10 15, 20 mg/mL in methanol) ($R^2 = 0.9992$) was used. Results were calculated and expressed as mg gallic acid equivalent (GAE)/g sample, in dry basis (db).

2.7. Total anthocyanins analysis

The method used was described by Fuleki and Francis (1968) and further detailed by Awika, Rooney, and Waniska (2004). The absorbance of sorghum extract samples were read at 480 nm in a spectrophotometer (Hitachi UV–Visible 1100). The concentrations of 3-deoxyanthocyanins were calculated based on the absorbance of luteolinidin at 480 nm using the Lambert-Beer's Law: $A = \varepsilon CL$, where A is the absorbance at 480 nm, ε is the molar extinction coefficient (ε) of luteolinidin, C is sample concentration, L is the pathlength, which is 1 cm. The total anthocyanins content was calculated based on the formula $C\ (mol/L) = A/\varepsilon$. The mg luteolinidin equivalent (LE) of a sample = $A/\varepsilon \times 103 \times 270 \times$ dilution factor, where 270 is the molecular weight of luteolinidin and molar extinction coefficient (ε) of luteolinidin used was 29,157 (Njongmeta, 2009). Results were expressed as mg luteolinidin equivalents (LE)/g sample, on dry basis (db).

2.8. Condensed tannin analysis

The content of condensed tannins was determined only in the genotype SC319 because the genotype TX430 does not contain a pigmented testa and thus does not have any significant amount of condensed tannins (Dykes et al., 2005).

The vanillin-HCl method described by Price, Van Scoyoc, and Butler (1978) was used. For the extraction, 0.15 g of sorghum flour was mixed with 8 mL of 1% HCl in methanol. The tubes remained in a water bath (30 °C) and were agitated in a vortex 3 times at an interval of 20 min. The extracts were centrifuged for 15 min and then one aliquot (1 mL) of the supernatant was added to 5 mL vanillin reagent (0.01 g/mL vanillin in methanol mixed with equal volume 8% HCl in methanol) and allowed to react for 20 min at 30 °C. A blank was prepared under the same reaction condition by reacting 1 mL the same aqueous sample with 5 mL 4% HCl in methanol. Absorbance was read at 500 nm in a spectrophotometer (Hitachi UV–Visible 1100) and blank for each sample was subtracted. Serial concentrations of catechin (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL in methanol) were used as standards. Results were calculated and expressed as mg catechin equivalent (CE)/g sample, in dry basis.

2.9. Color analysis

Color measurements of the sorghum grains, flours and bran were obtained using a Minolta CR-410 Colorimeter (Osaka, Japan). The results were expressed in CIELAB space (L^* a^* b^*) color coordinates.

2.10. Retention (%) of compounds at the end of storage

The calculation of the apparent retention of the 3-deoxyanthocyanins, total anthocyanins, total phenols and tannins, at the end of storage (180 days), was performed according to the equation bellow:

$$Retention\ (\%) - [Content\ at\ 180\ days\ (T180) \div Content\ at\ time\ zero\ (T0)] \times 100$$

2.11. Statistical analysis

The effects of the type of the product, the temperature (4, 25 and 40 °C) and the storage time (6 months) on the color and content of total anthocyanins, 3-deoxyanthocyanins, total phenols and condensed tannins of the two sorghum genotypes were evaluated using a completely randomized design, in a $2 \times 3 \times 4$ factorial scheme (2 types of products × 3 temperatures × 4 storage times). For 3-DXAs analysis it was used a $2 \times 3 \times 3$ factorial (2 types of products × 3 temperatures × 3 storage times). The genotypes were analyzed separately. The data were analyzed by analysis of variance (ANOVA) and the averages of three replicates were submitted to the Tukey test at 5% of probability. Statistical analysis was done using the SISVAR computational model (Ferreira, 2003).

3. Results and discussion

3.1. 3-Deoxyanthocyanins (3-DXAs) contents

Because the genotypes SC 319 (grown in Minas Gerais, Brazil) and TX 430 (grown in Texas, USA) were cultivated in different conditions, the statistical analysis was performed separately and the results were presented in two individual Tables (Tables 1 and 2).

In the case of the genotype SC 319 the statistical analysis showed significant difference only on type of product (grain and flour) and on storage time (0, 60 and 180 days). There was no effect of the temperature on both products during storage, thus, the means were compared only between grain and flour and among the storage times (Table 1).

It was observed difference between grains and flour only in the luteolinidin and apigeninidin contents at the beginning of storage, with higher levels in the grains (Table 1). However, these contents were not significantly different from the 60th to the 180th day. So, in the first 60 days, there was a greater loss of 3-DXAs in the grains compared to the flour. This information is useful for food processing industries because they can have grain and flour with the same content of these anthocyanins even after 180 days of storage and the use of flour is more practical.

The 3-deoxyanthocyanins (luteolinidin, apigeninidin, 5-methoxyluteolinidin and 7-methoxyapigeninidin) contents of the genotype SC 319 on time zero were between 43 and 59% lower than those found by Cardoso et al. (2015) in the same genotype flour (103.6, 64.8, 120.0 and 54.0 μg/g for luteolinidin, apigeninidin, 5-methoxyluteolinidin and 7-methoxyapigeninidin, respectively). The variances observed in the two studies may be because they were planted in different locals in Brazil: Nova Porteirinha, MG in Cardoso et al. (2015) study and Sete Lagoas, MG in the present study. Hayes and Rooney (2014) also detected significant effects of the interaction of genotypes × environments on total phenols, condensed tannins and 3-deoxyanthocyaninis contents of six black grain sorghum hybrids grown in six Texas environments (three locations and two years).

No difference ($p > 0.05$) among temperatures (4, 25 and 40 °C) was detected for all 3-DXAS contents in the genotype SC 319, which confirms the statements of Yang, Dykes, and Awika (2014)

Table 1
3-Deoxyanthocyanins contents (luteolinidin, 5-methoxyluteolinidin, apigeninidin and 7-methoxyapigeninidin) of the sorghum genotype SC319 during storage at three temperatures.[A,B]

3-Deoxyanthocyanidins	Storage time	Grain					Flour				
		Storage temperature									
	Days	4 °C	25 °C	40 °C	Mean		4 °C	25 °C	40 °C	Mean	
Luteolinidin	0	59.6	59.6	59.6	59.6	aA	51.7	51.7	51.7	51.7	aB
	60	50.5	40.4	37.5	42.8	bA	42.1	42.5	44.0	42.9	bA
	180	38.9	41.1	32.6	37.5	bA	42.0	41.0	37.7	40.3	bA
	Retention[C] (%)				62.9					77.9	
5-Methoxyluteolinidin	0	52.3	52.3	52.3	52.3	aA	49.5	49.5	49.5	49.5	aA
	60	38.7	36.4	38.9	38.0	bA	37.3	38.8	38.3	38.1	bA
	180	31.8	36.4	30.9	33.0	bA	35.8	36.6	35.1	35.8	bA
	Retention[C] (%)				63.1					72.3	
Apigeninidin	0	29.6	29.6	29.6	29.6	aA	25.2	25.2	25.2	25.2	aB
	60	18.7	17.8	18.2	18.2	bA	18.5	19.1	19.8	19.1	bA
	180	18.6	18.0	13.3	16.6	bA	19.7	18.1	16.2	18.0	bA
	Retention[C] (%)				56.1					71.4	
7-Methoxyapigeninidin	0	22.6	22.6	22.6	22.6	aA	21.6	21.6	21.6	21.6	aA
	60	16.3	15.2	15.6	15.7	bA	16.0	16.0	15.9	16.0	bA
	180	13.4	14.5	14.0	13.9	bA	15.0	15.8	13.9	14.9	bA
	Retention[C] (%)				61.5					69.0	

Means followed by the same lower case letters on the same column (among storage times) and means followed by the same upper case letters on the same row (between grain and flour) do not differ significantly by the Tukey test ($p \leqslant 0.05$).
[A] Results are expressed as μg/g dry matter.
[B] Means of three replicates.
[C] % of retention from time 0 to 180.

Table 2
3-Deoxyanthocyanins contents (luteolinidin, 5-methoxyluteolinidin, apigeninidin and 7-methoxyapigeninidin) of the sorghum genotype TX430 during storage at three temperatures.[A,B]

3-Deoxyanthocyanidins	Storage time	Bran								Flour							
		Storage temperature								Storage temperature							
	Days	4 °C		25 °C		40 °C		Mean		4 °C		25 °C		40 °C		Mean	
Luteolinidin	0	844.0	aA	844.0	aA	844.0	aA	844.0	α	91.8	aA	91.8	aA	91.8	aA	91.8	β
	60	683.3	bA	663.6	bA	606.1	bB	651.0	α	70.9	aA	70.7	aA	52.2	aA	64.6	β
	180	604.1	cA	620.9	bA	592.1	bA	605.7	α	71.7	aA	66.8	aA	63.3	aA	67.3	β
	Retention[C] (%)							71.8								73.3	
5-Methoxyluteolinidin	0	919.1	aA	919.1	aA	919.1	aA	919.1	α	45.8	aA	45.8	aA	45.8	aA	45.8	β
	60	734.7	bA	675.6	bA	602.0	bB	670.7	α	41.1	aA	39.4	aA	27.5	aA	36.0	β
	180	639.0	cA	661.4	bA	613.5	bA	637.9	α	38.7	aA	39.9	aA	31.6	aA	36.7	β
	Retention[C] (%)							69.4								80.1	
Apigeninidin	0	173.8	aA	173.8	aA	173.8	aA	173.8	α	92.2	aA	92.2	aA	92.2	aA	92.2	β
	60	133.5	bA	127.1	bAB	113.8	bB	124.8	α	67.9	bA	66.0	bA	48.0	bB	60.6	β
	180	116.6	bA	123.8	bA	110.6	bA	117.0	α	69.7	bA	64.9	bA	58.1	bA	64.2	β
	Retention[C] (%)							67.3								69.7	
7-Methoxyapigeninidin	0	180.6	aA	180.6	aA	180.6	aA	180.6	α	45.4	aA	45.4	aA	45.4	aA	45.4	β
	60	150.8	bA	140.8	bAB	127.0	bB	139.5	α	37.0	aA	33.9	aA	25.9	aA	32.2	β
	180	146.8	bA	131.8	bA	127.2	bA	135.2	α	36.6	aA	35.0	aA	32.2	aA	34.6	β
	Retention[C] (%)							74.9								76.2	

Means followed by the same lower case letters on each column (among storage times) and the same upper case letters on each row (among storage temperatures) do not differ significantly by the Tukey test ($p \leqslant 0{,}05$).
Means followed by different Greek letters in the same row (between bran and flour) differ statistically by the Tukey test ($p \leqslant 0.05$).
[A] Results are expressed as μg/g dry matter.
[B] Means of three replicates.
[C] % of retention from time 0 to 180.

that these anthocyanins are very thermostable (Table 1). On the other hand, the time of storage influenced these compounds in the grains and flour, except in the 5-methoxyluteolinidin content in the flour (Table 1). There was a significant reduction ($p \leqslant 0.05$) in all 3-DXAs contents in grains and sorghum flour, from T0 (time zero) to T60. However, in both matrices, after 60 days, the four compounds remained stable for 180 days (Table 1). The decrease in the total anthocyanins content during storage may be due to degradation of them by oxidation. According to Cavalcanti, Santos, and Meireles (2011) many factors affect the color and stability of these compounds, as pH, heat, light, the presence of other phenolic compounds, enzymes, metal ions, sugars, ascorbic acid, oxygen among others. As the sorghum flours, grains and bran were stored for 180 days, it is possible that one of these factors have had an effect on the stability of sorghum anthocyanins.

Although there were significant losses in the 3-DXAs contents, sorghum grains and flours had high retention of their contents, respectively, 63.1 and 72.3% of 5-methoxyluteolinidin, 56.1 and 71.4% of apigeninidin, and 61.5 and 69.0% of 7-methoxyapigeninidin, even when they were stored at 40 °C

during 180 days (Table 1). According to Patras, Brunton, O'Donnell, and Tiwari (2010) the degree of degradation and stability depend on the severity of time and temperature. In this study, the temperature of 40 °C did not affect significantly the 3-DXAs contents, exhibiting the same behavior found at temperatures 4 and 25 °C.

It was not found any study on retention of sorghum 3-DXAs during storage, however, Cardoso et al. (2014) evaluated the effects of extrusion and dry heat in a conventional oven (DHCO, 121 °C, 25 min) on total phenols of grains of the SC 319 and two other genotype, and verified that the 3-deoxyanthocyanidins were stable in DHCO but were susceptible to extrusion cooking.

In the case of the genotype TX 430, the ANOVA showed significant effect of the type of product (bran or flour), the storage time and the interaction between these factors on the contents of all 3-DXAs (Table 2).

It was observed, from beginning to the end of the storage, that the luteolinidin, apigeninidin and their methoxylated derivatives contents were higher in the sorghum bran compared to the flour. The concentrations of luteolinidin and 5-methoxyluteolinidin were, respectively, about 10 and 20 times higher in the sorghum bran than in the flour. The apigeninidin and 7-methoxyapigeninidin were about 2 and 4 times, respectively, higher in the bran (Table 2). This result was expected since these compounds are concentrated in the pericarp of the grain (Moraes et al., 2015). Awika et al. (2004) have already reported the expressiveness of 3-DXAs present in bran of the black sorghum, whose contents were 3–4 times higher than that in the whole grains and two times higher than in the red and brown sorghum brans.

There was effect of the storage time ($p \leqslant 0.05$) in the first 60 days, with reduction of all 3-DXAs levels in the bran, but, from 60 to 180 days, the concentrations remained stable. The retentions of these compounds in bran were 71.8% (luteolinidin), 69.4% (5-methoxyluteolinidin), 67.3% (apigeninidin) and 74.9% (7-methoxyapigeninidin) from the start time to the end of storage. This result will be useful to the pharmaceutical industries that may use the sorghum bran to extract anthocyanins to use in nutraceutical products (Table 2).

The 3-DXAS contents (except apigeninidin) of the genotype TX430 flour were less influenced by the storage time. These levels were statistically similar from the time zero to 180th day with retentions of 73.3, 80.1 and 76.2% of luteolinidin, 5-methoxyluteolinidin and 7-methoxyapigeninidin, respectively (Table 2). This result will be helpful for the end consumer that can use the sorghum flour in various food preparations taking advantage of their functional potential.

In most cases, the temperature did not affect the 3-DXAs contents in the sorghum bran and flour, except in the first 60 days of storage in the sorghum bran (all 3-DXAs) and in the apigeninidin content in the flour (Table 2), which the temperature of 40 °C caused the greatest loss of these compounds. Devi et al. (2012) studied the anthocyanins stability of red sorghum bran extracts in eight different temperature (from 0 to 70 °C), light (presence or absence) and pH (from 1.1 to 9.9) and, corroborating the present study, they also found good stability of them against these factors, although they did not study the retention of these compounds during the storage. Yang et al. (2014) evaluated the 3-DXAS stability to 95 °C and 121 °C in different intervals of time (30 min, 1 h and 2 h) and at pH varying from 1 to 6. They also found good thermal stability of the extracts obtained at 95 °C/2 h and 121 °C/30 min and in low acidity condition. The high stability at different temperatures and pH values associated with the 3-deoxyanthocyanins of the sorghum bran showed a great potential of these compounds to be explored as food colorant.

Although the genotypes SC319 (brown pericarp) and TX430 (black pericarp) have not been compared together in the statistical analysis, it was observed that apigeninidin contents were almost four times higher and the luteolinidin and 7-methoxyapigeninidin were approximately two times higher in the TX430 flour compared to SC319 flour. Only the 5-methoxyluteolinidin content was similar in both genotypes. This result is consistent with that reported by Dykes et al. (2005) that evaluated the effects of plant color, pericarp thickness, pigmented testa, and spreader genes on total phenols, condensed tannins, flavan-4-ols, and anthocyanins levels of 13 sorghum genotypes. The authors found that sorghums with a black pericarp had higher levels of flavan-4-ols and anthocyanins than the other varieties analyzed.

3.2. Total anthocyanins

There was no significant effect ($p > 0.05$) of the storage temperatures (4, 25 and 40 °C) and the temperature x storage time interaction on the total anthocyanins content. The differences were verified only regarding storage time for the genotype SC319 and for storage time, type of product and type of product × storage time interaction for the genotype TX430. So, the average of total anthocyanins content were compared only among storage time, regardless of temperatures (Table 3).

Although the genotypes SC319 (brown pericarp) and TX430 (black pericarp) have not been compared together in the statistical analysis, it was observed that the flour of the genotype SC319 had almost three times less anthocyanins than the genotype TX430 Black corroborating the results found to the 3-deoxyanthocyanins contents in this present study. This agrees with the results of Dykes et al. (2005) who found that the sorghums with a black pericarp had higher levels of total anthocyanins than the other varieties analyzed.

It was observed that the contents of total anthocyanins in the flours of both genotypes and in the grains of the genotype SC319 remained stable for 120 days. The anthocyanins in the bran showed lower storage stability than the flour and grains and reduced from T0 to T60. However, the total anthocyanin retention at the end of storage was high in all products in the following order: flour of TX430 (96.8%) > grain of SC319 (93.8%) > flour of SC319 (88.4%) > bran of TX430 (84.6%) (Table 3).

This study is the first found in the literature, which evaluated the effect of storage temperatures and storage time on total anthocyanins levels in sorghum. Mirsaeedghazi, Emam-Djomeh, and Ahmadkhaniha (2014) studied the stability of total anthocyanins and five major anthocyanins (Cyanidin 3-glucoside, Cyanidin 3,5-diglucoside, Delphinidin 3-glucoside, Pelargonidin 3-glucoside and Pelargonidin 3,5-diglucoside) in pomegranate juice stored at −25 °C. The results showed that Pelargonidin 3,5-diglucoside had the greatest decrease and that ellagic acid decreased by 15%. Total anthocyanin content of pomegranate juice decreased by 11%. Hellström, Matilla, and Karialainen (2013) monitored the stability of structurally different anthocyanins in several berry juices during storage at different temperatures. In all studied juices, half-life of anthocyanins was much shorter at room temperature than at cold storage. Reque et al. (2014) determined the changes occurring during storage of blueberries (*Vaccinium* spp.) juice at 4 °C for 10 days and of blueberries fruits at −18 °C for 6 months. There were significant losses of anthocyanins both in the refrigerated juice (83%) and in the frozen fruits (59%).

The high stability of anthocyanins observed in the flours, grain and bran of sorghum during 180 days of storage, even when such products are exposed to temperatures of 25–40 °C, may be explained to the absence of an oxygen atom in the C3 position (Awika et al., 2004; Clifford, 2000). This structure provides greater stability to the sorghum anthocyanins than those found in fruits and vegetables, which require low temperatures for their

Table 3
Total anthocyanins content in the sorghum genotypes SC319 and TX430 during storage at three temperatures.[A,B]

Genotype	Product	Storage temperature	Storage time – T (days)								Retention[C] (%)
			0		60		120		180		
SC319	Flour	4 °C	0.43		0.42		0.40		0.39		
		25 °C	0.43		0.40		0.39		0.39		
		40 °C	0.43		0.40		0.38		0.36		
		Mean	0.43	a	0.41	ab	0.39	ab	0.38	b	88.4
	Grain	4 °C	0.43		0.43		0.43		0.42		
		25 °C	0.43		0.43		0.41		0.39		
		40 °C	0.43		0.43		0.41		0.40		
		Mean	0.43	a	0.43	a	0.42	ab	0.40	b	93.8
TX 430 Black	Flour	4 °C	1.25		1.23		1.19		1.21		
		25 °C	1.25		1.23		1.20		1.20		
		40 °C	1.25		1.19		1.23		1.21		
		Mean	1.25	aB	1.22	aB	1.21	abB	1.21	aB	96.8
	Bran	4 °C	7.46		6.61		6.65		6.43		
		25 °C	7.46		6.93		6.30		6.47		
		40 °C	7.46		6.51		6.19		6.04		
		Mean	7.46	aA	6.68	bA	6.38	bA	6.31	bA	84.6

Means followed by the same lower case letters in the row (among storage times) and the same upper case letters in the column (between products of each genotype) are not statistically different by the Tukey test ($p \leqslant 0.05$).
[A] Results are expressed as mg luteolinidin equivalents (LE)/g sample on dry basis (db).
[B] Means of three replications.
[C] % of retention from time 0 to 180.

conservation (Bakhshayeshi, Khayami, Heidari, & Jamei, 2006; Bolivar & Cisveros-Zevallos, 2004).

It has been recognized that anthocyanin-rich extracts might be used as food additives. However, many factors influence the stability of anthocyanins and heat and light can destroy them during processing of fruits and vegetables (Pietta, Minoggio, & Bramati, 2003). Thus, the result of this work shows that sorghum may be an important source of natural food additives with health benefits.

The high stability of sorghum anthocyanins, observed in storage at temperatures between 4 and 40 °C for a period of 180 days, suggests that sorghum grains as well as sorghum flour and bran can be stored at room temperature, and thus, saving energy. This is an important result considering the diversity of climates that occur in the different regions of the world, especially in the five regions of Brazil.

3.3. Condensed tannins

The effect of the storage on the content of total condensed tannins was analyzed only in the genotype SC 319 because the genotype TX430 did not show any significant amount of tannins (Dykes et al., 2005).

The ANOVA showed significant differences for the storage time, storage temperature and storage temperature × storage time interaction for the tannin contents of sorghum flour and grains (Table 4). Thus, the treatments were analyzed within each temperature.

The condensed tannins content of sorghum flour samples stored at 4 and 25 °C showed no significant differences during storage. Stability at these temperatures was maintained for 180 days with high retention at the end of this period, 85.3% at 4 °C and 83.0% at 25 °C. However, the flour stored at 40 °C resulted in lower levels during storage with retention of 56.6%, despite stability was observed from the initial time (T0) until the 60th day. This lower levels of tannins, observed during storage at 40 °C, may be due to interactions of tannins with proteins and carbohydrates (e.g. starch), which decrease tannin extractability (Barros et al., 2012).

The content of tannins in the grains was better preserved at 4 °C. At this temperature the retention was 79.1% in the 180th day, this result was slightly lower compared to the flour at the same temperature (85%). Temperatures of 25 °C and 40 °C influenced tannin content in the grain since the time 0 until 180 days, with significant decreases during storage. After 180 days, the retentions at 25 °C and 40 °C were 76.9 and 72.8%, respectively, better than the retention on the flour at 40 °C (56.6%).

The tannin retention (all three temperatures) of the genotype SC319, after 180 days of storage was approximately 75% for flour and 76% for grain, less stable than anthocyanins (88.4% and

Table 4
Tannins content in sorghum flour and grain of the genotype SC319 during storage at three temperatures.[A,B]

Product	Storage temperature	Condensed tannins								
		Storage time – T (days)								Retention[C] %
		0		60		120		180		
Flour	4°	16.96	aA	14.64	aAB	16.22	aAB	14.46	aB	85.3
	25°	16.96	aA	15.65	aAB	14.71	aAB	14.08	aB	83.0
	40°	16.96	aA	15.18	aA	10.80	bB	9.60	bB	56.6
Grain	4°	17.23	aA	17.71	aA	16.77	aA	13.62	aB	79.1
	25°	17.22	aA	15.75	abA	14.86	abAB	12.54	aB	76.9
	40°	17.22	aA	15.26	bAB	14.44	bB	13.25	aB	72.8

Means followed by the same lower case letters on the same column (among storage temperatures) and means followed by the same upper case letters on the same row (among storage times) do not differ significantly by the Tukey test ($p \leqslant 0.05$).
[A] Results are expressed as mg catechin equivalent (CE)/g sample on dry basis (db).
[B] Means of three replications.
[C] % of retention in 180 days.

93.8% for flour and grain respectively) and total phenols compounds (86.7% and 86.8% for flour and grain, respectively).

Oliveira, Silva, Pinho, and Abreu (2011) evaluated the tannin contents in sorghum with high tannins, stored after drying in artificial dryers at temperatures of 35 °C, 45 °C and 35/45 °C until water content was 12%. After drying, the seeds were stored in a cold, dry chamber for 0, 3 and 6 months. The tannin concentration reduced from time zero to the third month and increased after six months of storage.

3.4. Total phenols

The results showed no effect of the type of product (flour and grain), storage temperature and the interaction of the factors ($p > 0.05$) on the phenols contents of the sorghum genotype SC319 samples during storage. In both matrices, significant differences ($p \leqslant 0.05$) were observed only for the storage time. Cardoso et al. (2014) also found high stability of the total phenols to dry heat (retention of 106.3%) and low stability with wet heat.

The phenols were stable for 120 days in the flour and for 60 days in the grains (Table 5) and showed high retention in both products (about 86%) at the end of storage.

Concerning the genotype TX430, neither the time nor the storage temperature influenced the phenols content. There was variation only between the type of product (flour and bran). The bran showed about 2 times more phenols than the flour at the beginning of storage. This result was expected since the phenolic compounds are concentrated in the pericarp of the sorghum grain. Moraes et al. (2015) evaluated the total phenols in sorghum bran and flour (whole and decorticated) of the genotype SC21 and found 8.33 mg GAE/g in the whole flour, less than the present work for both flours analyzed (11.13 and 11.73 mg GAE/g), 1.44 mg GAE/g in the decorticated flour and 31.95 mg GAE/g in the bran, more than the bran of the genotype TX430 (25.04 mg GAE/g). Although the bran showed higher phenol contents than the flour, at the end of storage the flour had 100% of these compounds retention, higher than the bran (89.4%).

The phenols contents of the both flours genotypes were similar. This fact may be because although the TX430 has high levels of anthocyanins, the genotype SC 319 has high levels of condensed tannins and both compounds contribute to the total phenols contents.

The phenols content kept stable in sorghum flour and grains from the initial time (T0) until the 120th day of storage. Although the levels had reduced at the end of 180 days, the retentions of these compounds were high, 87.6% in flour and 93% in grains even at a temperature of 40 °C. These results are similar to those found to the total anthocyanins.

Mirsaeedghazi et al. (2014) studied the total phenolic contents in pomegranate juice and verified stability when stored for 5, 10, 15 and 20 days at −25 °C. The authors concluded that, after 15 days' storage, the total phenolic contents decreased 29%.

Sikwese (2005) investigated the effect of the storage temperature and the length of storage, in the total phenols content of a freeze-dried crude phenolic extract (CPE) of two Malawian sorghums. Although CPE samples stored at −20 °C had significantly higher levels of total phenols than those stored at 25 °C during some days of storage, the author concluded that the storage time was the major factor influencing the levels of total phenols, corroborating this study.

3.5. Color – L^*, a^* and b^* values

It was observed significant difference ($p \leqslant 0.05$) for L^*, a^* and b^* values among type of products, storage time and type of product × storage time interaction. No significant difference ($p > 0.05$) was detected for the storage temperature and for temperature × storage time interaction of both sorghum genotypes samples. These results indicated that the storage temperatures (4, 25 and 40 °C) did not interfere in the color of the flour and grain of the genotype SC319 and in the flour and bran of the genotype TX430. However, the storage time caused alterations on color of all products during storage (Table 6).

All samples had color stability from the initial time to 120 days of storage, based on L^*, a^* and b^* values (Table 6). Starting this time to the end of storage there was an increase in L^* value, which means that they became less dark in color. All samples had positive a^* and b^* values, which means that they were more red than green and more yellow than blue. There was an increase in a^* and b^* values from 120 to 180 days in all samples, which means an intensification of the red and yellow color (Table 6). In all cases, the a^*

Table 5
Total phenols content in the sorghum genotypes SC319 and TX430 during storage at three temperatures.[A,B]

Genotype	Product	Temperature	Storage time – T (days)								Retention[C] %
			0		60		120		180		
SC 319	Flour	4 °C	11.13		10.79		10.34		10.29		
		25 °C	11.13		10.75		9.72		8.78		
		40 °C	11.13		10.90		10.28		9.89		
		Mean	11.13	aA	10.81	aA	10.11	abA	9.65	bA	86.73
	Grain	4 °C	11.17		10.72		10.27		10.08		
		25 °C	11.17		10.57		10.38		9.95		
		40 °C	11.17		10.36		9.07		9.07		
		Mean	11.17	aA	10.55	abA	9.91	bA	9.70	bA	86.84
TX 430 Black	Flour	4 °C	11.73		11.53		11.36		11.12		
		25 °C	11.73		11.67		11.09		10.96		
		40 °C	11.73		10.52		10.15		13.10		
		Mean	11.73	aB	11.24	aB	10.87	aB	11.73	aB	99.97
	Bran	4 °C	25.04		24.66		23.3		23.1		
		25 °C	25.04		22.85		22.52		22.01		
		40 °C	25.04		22.77		22.58		22.06		
		Mean	25.04	aA	23.43	aA	22.8	aA	22.39	aA	89.42

Means followed by the same lower case letters in the row (among storage times) and the same upper case letters in the column (between products of each genotype) are not statistically different by the Tukey test ($p \leqslant 0.05$).

[A] Results are expressed as mg gallic acid equivalent (GAE)/g sample on dry basis (db).

[B] Means of three replications.

[C] % of retention from time 0 to 180.

Table 6
Colorimetric analysis (L^*, a^* and b^* values) of sorghum genotypes SC319 and TX430 during storage at three temperatures.[A]

Genotype	Variable	Storage time	Flour					Grain				
			Storage temperature					Storage temperature				
			4 °C	25 °C	40 °C	Mean		4 °C	25 °C	40 °C	Mean	
SC319	L^*	0	47.9	47.9	47.9	47.9	bA	23.4	23.4	23.4	23.4	bB
		60	48.2	48.9	48.9	48.7	bA	25.7	24.4	24.2	24.8	abB
		120	49.9	49.9	49.5	49.7	bA	27.2	24.9	26.3	26.1	abB
		180	68.1	67.2	67.5	67.6	aA	29.4	30.1	27.8	29.1	aB
	a^*	0	5.4	5.4	5.4	5.4	bB	11.1	11.1	11.1	11.1	bA
		60	5.1	5.2	5.3	5.2	bB	10.9	12.0	13.3	12.0	bA
		120	5.5	5.4	5.5	5.5	bB	10.9	13.1	13.9	12.6	abA
		180	5.9	6.0	6.0	6.0	aB	14.2	14.2	14.2	14.2	aA
	b^*	0	9.4	9.4	9.4	9.4	bB	18.1	18.1	18.1	18.1	aA
		60	9.6	9.6	10.0	9.7	bB	19.7	20.5	18.5	19.5	aA
		120	10.1	9.3	9.3	9.6	bB	20.6	21.0	19.1	20.2	aA
		180	12.1	11.7	12.0	11.9	aB	20.8	22.8	20.6	21.4	aA
			Flour					Bran				
TX430 Black	L^*	0	45.6	45.6	45.6	45.6	bA	36.5	36.5	36.5	36.5	bB
		60	46.3	46.5	45.2	46.0	bA	35.8	36.2	36.6	36.2	bB
		120	46.4	46.8	46.0	46.4	bA	40.7	35.6	37.4	37.9	bB
		180	65.5	65.2	64.6	65.1	aA	53.2	53.1	53.5	53.2	aB
	a^*	0	6.6	6.6	6.6	6.6	bB	9.3	9.3	9.3	9.3	bA
		60	6.5	6.5	6.5	6.5	bB	9.0	9.1	9.1	9.1	bA
		120	6.6	6.7	6.5	6.6	bB	9.3	8.9	9.2	9.1	bA
		180	7.6	8.2	7.9	7.9	aB	11.3	11.3	11.1	11.2	aA
	b^*	0	7.3	7.3	7.3	7.3	bB	8.5	8.5	8.5	8.5	bA
		60	7.0	7.0	7.0	7.0	bB	8.3	8.4	8.7	8.5	bA
		120	7.1	7.1	7.1	7.1	bB	8.5	8.3	9.0	8.6	bA
		180	8.9	9.0	9.0	9.0	aB	10.8	10.6	11.2	10.8	aA

Means followed by the same lower case letters on the same column (among storage times) and means followed by the same upper case letters on the same row (between products of each genotype) do not differ significantly by the Tukey test ($p \leqslant 0.05$).
[A] Means of three replications.

and b^* value increased as the L^* value increased corroborating the results showed by Dykes et al. (2005).

The sorghum flours had higher L^* value than the grains (SC 319) and bran (TX430) from the time zero to 180 days. This result is consistent because the grain pericarps of the SC319 (brown) and TX430 (black) are darker than their endosperms (cream), so the bran and grains are darker than the flour which is a mixture of the pericarp and endosperm.

The color is visually one of the most attractive quality attribute for consumers and directly influences food acceptance (Saldaña, Siche, Luján, & Quevedo, 2013). The whitening observed after 180 days in flours, grains (SC 319) and bran probably would not affect the final quality of the final products because the genotypes studied are naturally dark.

4. Conclusions

The storage temperature did not affect the color, the total anthocyanins and total phenols contents and most of the 3-deoxyanthocyanins contents of the sorghum genotypes SC319 and TX430; however, most of them were influenced by the storage time. Although there was a reduction in all phenolic compounds contents of both genotypes in the first 60 days, after 180 days of storage they showed retention of almost 90% of total anthocyanins and phenols and above 60% of luteolinidin and 5-methoxyluteolinidin, 7-methoxyapigeninidin and 50% to apigeninidin and tannins.

The high retention observed in most of the analyzed phenolic compounds stored at temperatures between 4 and 40 °C after 180 days, suggest that sorghum grains as well as sorghum flour and bran can be stored at room temperature, and thus, saving energy. This is an important result considering the diversity of climates that occur in the different regions of the world.

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