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Original article

Effect of light, food additives and heat on the stability of sorghum 3-deoxyanthocyanins in model beverages

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Abstract This work aimed to evaluate the stability of sorghum 3-deoxyanthocyanins (DXA) in model beverages (pH 3.5) elaborated with crude sorghum phenolic extract, containing ascorbic acid and sulphite, under fluorescent light exposure and subjected to heat treatment. There was no significant difference in the DXA degradation during storage under light exposure (24.16%) and absence of light (20.72%). DXA degradation did not differ in the presence of ascorbic acid during storage under light exposure (23.99–25.38%) and absence of light (19.87–21.74%). The addition of sulphite caused an initial bleaching reaction, but as a reversible reaction, the anthocyanin content was higher on the last day of storage compared to the first day. There were no significant differences in total anthocyanin content of all treatments subjected to the heat treatment (80 °C for 5 and 25 min). Thus, crude DXA are very stable under light, additives and heat, and may be useful as natural food colourants.

Keywords Natural colourants, bioactive compounds, sulphite, ascorbic acid, flavonoids, *Sorghum bicolor* L..

Introduction

Anthocyanins are flavonoids and make up the largest group of water-soluble pigments found in the plant kingdom, ranging particularly from red, blue and purple in many fruits and vegetables (Fallah *et al*., 2020). These pigments have been extensively studied due to their bioactive properties and potential for application as natural food colourants (Castañeda-Ovando *et al*., 2009), whose demand is increasing because of their health benefits and consumer interest for cleaner labels (Amchova *et al*., 2015; Rodriguez-Amaya, 2019; Fallah *et al*., 2020). Anthocyanins have high antioxidant capacity and, therefore, can be used to inhibit oxidation reactions (Diaconeasa *et al*., 2015). Recent studies have demonstrated that anthocyanins have a cytotoxic effect on cancer cells and can help control diabetes and cardiovascular diseases (Jennings *et al*., 2012; Kim *et al*., 2015; Maciel *et al*., 2018).

Despite their use as natural food colourants, anthocyanins still have some limitations, since they are susceptible to degradation by factors such as light, pH, heat and enzymes (Francis & Markakis, 1989). Due to the ionic nature of anthocyanins, changes in the structure of the molecule can occur according to the predominant pH (Mazza & Brouillard, 1990). Under acidic conditions (pH <2), anthocyanin exists mainly in the form of the flavylium cation, with a red colour. The increase in pH values leads to rapid loss of the proton, producing forms of quinonoidal base with blue or violet colouration. At the same time, the hydration of the flavylium cation occurs, generating carbinol or pseudobase, which slowly reaches equilibrium with the colourless chalcone (Iacobucci & Sweeny, 1983). Another factor that affects the stability of anthocyanins is their copigmentation with other compounds (e.g. phenolic acids, flavonoids, metals), which is the main mechanism of colour stabilisation in plants and it is pH dependent (Mazza & Brouillard, 1990; Castañeda-Ovando *et al*., 2009).

The presence of food additives also affects the stability of anthocyanins. Sulphites used in the food industry produce discolouration in anthocyanins (Cavalcanti *et al*., 2011). The bleaching reaction is generally reversible depending on the structure of the pigment involved, pH and oxygen availability in the solution (Timberlake & Bridle, 1967). Ascorbic acid is often used by the food industry; however, its use in products containing anthocyanins is limited since it significantly accelerates the destruction of these

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pigments in the presence of oxygen due to the oxidative degradation of ascorbic acid that produces many reactive degradation products (e.g. hydrogen peroxide) that interact with anthocyanins (Sondheimer & Kertesz, 1953; Cao *et al*., 2012). Thus, several research works have been directed to the search for alternative sources of this pigment with greater stability (Cavalcanti *et al*., 2011; Rodriguez-Amaya, 2019). Sorghum (Sorghum bicolor (L.) Moench) is a unique grain among the cereals, having high levels of a diverse range of phenolic compounds, including the 3-deoxyanthocyanins (DXA) (Girard & Awika, 2018).

The sorghum DXA comprise luteolinidin and apigeninidin, including their methoxylated derivatives, 5-methoxyluteolinidin and 7-methoxyapigeninidin (Awika & Rooney, 2004; Dykes & Rooney, 2006). These sorghum DXA do not have the hydroxyl group at the C-3 position (Dykes & Rooney, 2006). This characteristic improves their stability when compared to those commonly found in vegetables and fruits (Awika *et al*., 2005). Sorghum DXA showed higher colour retention than anthocyanins from fruits and vegetables when processed at 95°C/2 h and 121°C/30 min, and at pH variations (Yang *et al*., 2014; Akogou *et al*., 2018). Ojwang & Awika (2010) demonstrated that even after the bleaching effect of the sorghum DXA due to the addition of sulphite, they recovered partially or totally their colour within 14 and 21 days, at pH 3.0 and 1.8. Moreover, even in the presence of ascorbic acid, DXA showed low colour loss when compared to red cabbage anthocyanins (Ojwang & Awika, 2008). In addition to these chemical advantages, many studies have demonstrated the health benefits of the DXA, such as chemopreventive properties (Yang *et al*., 2009; Suganyadevi *et al*., 2013; Luo *et al*., 2018), which make them potential bioactive food ingredients.

However, there have been no studies on the stability of crude sorghum DXA in model beverage systems under light exposure. Furthermore, it is important to evaluate the stability of such anthocyanins in the presence of additives, in concentrations commonly found in foods, and subjected to heat treatment. Thus, the objective of this work was to evaluate the stability of crude sorghum DXA in model beverages containing ascorbic acid and sulphite, under fluorescent light exposure and subjected to heat treatment.

Materials and methods

Materials

Hydrochloric acid was purchased from Synth, sodium carbonate from Alphatec, potassium persulphate from Exôdo, and the other reagents were purchased from Sigma Aldrich: Folin–Ciocalteu, gallic acid, sodium carbonate, Trolox, DPPH, ABTS, ascorbic acid and metabisulphite of sodium.

The sorghum genotype SC 084, harvested in 2018, was supplied by Embrapa Milho e Sorgo, from Sete Lagoas, Minas Gerais. This genotype had the highest total anthocyanin content among 230 genotypes of a panel with high genetic variability evaluated by Embrapa Milho e Sorgo. These data are confidential (not published) and are stored in a database with restricted access to Embrapa Milho e Sorgo. Sorghum grains were kept under refrigeration (5 °C ± 1) after harvest until use.

To obtain the sorghum bran, the grains were decorticated in a NOGUEIRA CIMAG rice processing machine (rural model, series B-7, 760 rpm), then ground in a RETSCH ball mill (model MM200) and granulometry was standardised on a 500 μm sieve. Sorghum bran (approximately 10% of original grain weight) was stored in plastic bags under refrigeration (5 °C ± 1).

Obtention and characterisation of the crude sorghum phenolic extract

The extraction was carried out according to Barros *et al*., (2013) with modifications. Sorghum bran was mixed with 70% v/v acidified ethanol/water (pH 2) at the ratio of 1:100 of bran: solvent and kept under agitation (KLINE shaker, New Technique) at room temperature for 2 h. The resulting extract was centrifuged at 2,000 x ***g*** for 10 min (NT 815, New Technique) and the supernatant was rotary evaporated (Rotary evaporator IKA RV100), vacuum filtered using Whatman No.1 filter paper and quantitatively transferred to volumetric flasks. The final volume of crude sorghum phenolic extract was 2.5 L. Subsequently, it was stored in amber bottles under refrigeration (5 °C ± 1). Characterisation of the crude sorghum phenolic extract (total anthocyanins and phenolics, antioxidant capacity and colorimetric analysis) was done immediately after extraction.

Total anthocyanins

Total anthocyanins were determined only at pH 1 (Awika *et al*., 2004). The wavelength used was 480 nm. For the phenolic extract of sorghum bran, the extinction coefficient (ϵ) of luteolinidin (29,157 L/mol.cm), the major anthocyanidin, was used. The final result was expressed in mg of Luteolinidin equivalent (LE)/100 mL of extract.

Total phenolics

The total phenolic content was determined by the method proposed by Singleton & Rossi (1965). The absorbance of the crude sorghum phenolic extract was read on a spectrophotometer (UV-1601 PC, Shimadzu) at 760 nm. A standard curve of gallic acid was used and the results were expressed in mg of gallic acid equivalent per 100 mL of extract (mg GAE/100 mL).

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Antioxidant capacity

The antioxidant capacity of phenolic extracts was obtained using the ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate) and DPPH (2,2-diphenyl-1-picrylhydrazine) methods. The ABTS method described by Re *et al.*, (1999) was used. The DPPH antioxidant capacity was evaluated according to Kim *et al.*, (2002) with modifications. An aliquot (0.5 mL) of the phenolic extract was added to 3.5 mL of the DPPH solution and the absorbance recorded (UV-1601 PC, Shimadzu) after 1 h of reaction. Trolox was used as standard and the results were expressed as μmol Trolox equivalent (TE) per litre of extract (μmol TE/L) in both methods.

Colorimetric analysis

The colorimetric evaluation was performed using the Cielab system in ColorQuest XE colorimeter (Hunter Lab, Reston, VA) in which the values of the coordinates L* (luminosity), a* (intensity of red vs green) and b* (intensity of yellow vs blue) were read, using a 15 mL cuvette. The calculation of the parameters h* (hue) and c* (saturation) was performed, according to equations 1 and 2, respectively.

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$c^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

Stability study

The stability study was performed in two steps, described below. Model beverage systems were prepared using the crude sorghum phenolic extract in the presence of additives (sulphite and ascorbic acid), citrate buffer (pH 3.5) and preservative (potassium sorbate and sodium benzoate). For the light stability, the beverages were stored for 21 days under fluorescent light and analysed on days 0, 7, 14 and 21. For the heat stability, the analyses were performed at 80 °C, at times 0, 5 and 25 min.

The pH 3.5 has been chosen because it is a common pH used in beverages and provides good stability to the anthocyanins, which are more stable at lower pHs (Ojwang & Awika, 2008). The experiment time was based on a study carried out by Ojwang & Awika (2010), who evaluated the stability of some 3-deoxyanthocyanidins in the presence of sulphite.

Stability of sorghum anthocyanins in model beverages containing sulphite and ascorbic acid, under light exposure

The experimental design was followed according to Fig. S1. Crude sorghum phenolic extract was diluted in pH 3.5 citrate buffer and fractionated in five different treatments to obtain model beverage systems: (1) extract without additives (E_S); (2) extract + 0.002 g/100 mL of sodium metabisulphite (E_{Su1}); (3) extract + 0.004 g/100 mL of sodium metabisulphite (E_{Su2}); (4) extract + 0.015 g/100 mL of ascorbic acid (E_{Ac1}); (5) extract + 0.03 g/100 mL of ascorbic acid (E_{Ac2}). The volume added of crude phenolic extract per 100 mL of citrate buffer was 31.6 mL for the treatments E_S, E_{Ac1}, E_{Ac2} and 66.70 mL for the treatments E_{Su1} and E_{Su2}. Moreover, the amount of sorghum seeds needed to obtain 100 mL of beverage was approximately 9.6 g for the treatments E_S, E_{Ac1}, E_{Ac2} and 16.0 g for the treatments E_{Su1} and E_{Su2}. Concentrations of sodium metabisulphite (ISN 223) were used according to the maximum limit allowed for carbonated and non-carbonated soft drinks by RDC no 5, of January 15, 2007 (BRASIL, 2007). For ascorbic acid (ISN 300), concentrations were used according to the maximum limit allowed for refreshments and soft drinks by RDC no 4 and by the RDC no. 45, which establishes food additives authorised for use according to good manufacturing practices (BPF) in Mercosur (BRASIL, 1988). The absorbance was then controlled in order to obtain an initial absorbance between 0.8 and 0.9 at the maximum absorption length in a spectrophotometer (UV-1601 PC, Shimadzu). Finally, potassium sorbate and sodium benzoate were added at 0.1% w/v, as preservatives (Estupiñan *et al.*, 2011).

Treatments were stored in transparent glass bottles, hermetically closed, with the use of nitrogen gas, under the incidence of 40W fluorescent light, 2500 lux, corresponding to storage light, in TruVue 2 equipment (Datacolor), under room temperature (25 °C ± 1). As a control, treatments equal to the previous were stored in amber flasks, hermetically sealed, using nitrogen gas, in the absence of light. There was a single glass bottle, for each of the three repetitions of each treatment, each day, which was used on the day of the analysis and then discarded.

Stability of sorghum anthocyanins in model beverages containing sulphite and ascorbic acid subjected to heat treatment

The experimental design was followed according to Fig. S2. The different treatments obtained (E_S, E_{Su1}, E_{Su2}, E_{Ac1} and E_{Ac2}) were subjected to heat treatment in amber glass bottles immersed in a water bath (Marconi MA 120) at 80 °C for 0, 5 and 25 min. There was a single glass bottle, for each of the three repetitions of each treatment, each time, which was used at the time of the analysis and then discarded. After heat treatment, the bottles were transferred to a cold-water bath until they reached room temperature to perform the analysis. The heat treatment condition was selected since the binomial time and temperature (80 °C/3 min

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and 5 min) are often used in juice pasteurisation by the food industry (personal communication).

Analysis during the stability study

For the different treatments obtained, analysis of total anthocyanins and colour, as described previously, were performed. In addition, for the colorimetric analysis, the global colour difference (ΔE*) was calculated during the stability study, according to equation 3. Graphs of the Napierian logarithm (ln) of the initial absorbance/absorbance ratio versus time were constructed, and the degradation constant (k_d) was obtained through the slope. From the k_d (1/day), half-life values (days) were calculated in order to estimate the anthocyanins stability under light exposure, heat and presence of additives (Equation 4).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

$$t_{1/2} = \ln 2 / k_d \quad (4)$$

Statistical analysis

All analyses were performed in 3 repetitions. The data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) in a factorial design was used. For the study of the anthocyanins stability under light exposure, a 2-factor factorial design was used for the degradation kinetics (additives in 3 levels × storage condition in 2 levels), a 3-factor factorial design for the anthocyanins content variation and C* and h* colorimetric parameters (additives in 5 levels × condition of storage in 2 levels × time in 4 levels) and a 2-factor factorial design for the ΔE* (additives in 5 levels × storage condition in 2 levels). For the study of stability under heat treatment, a 2-factor factorial design was used for the variation of anthocyanins and for all colorimetric parameters (additives in 5 levels × time in 3 levels). Differences in means were compared using Tukey's post hoc test. All analyses adopted a significance level of 5% and were performed using the STATISTICA 13.3 software.

Results and discussion

Characterisation of the crude sorghum phenolic extract

Total anthocyanins and phenolic content of the crude sorghum phenolic extract were 12.0 ± 0.03 mg LE/100 mL and 238.98 ± 0.11 mg GAE/100 mL, respectively. The antioxidant capacity by ABTS method was 13.45 ± 0.15 μmol TE/L and by DPPH it was 68.88 ± 0.16 μmol TE/L. These values are higher than those found in some fruits such as jambolan and blackberry (Sari *et al*., 2012; Rigolon *et al*., 2020). This confirms that sorghum is a good source of anthocyanins and phenolic compounds. Moreover, it is important to emphasise that the sorghum genotype used is from Brazil and has the highest total anthocyanin content among those evaluated by Emprapa Milho e Sorgo (Embrapa's private database, not published).

Regarding the colorimetric analysis, the values of L* (lightness), a* (blue to yellow tones) and b* (green to red tones) were 49.25 ± 0.07, 33.87 ± 0.06 and 41.94 ± 0.08, respectively, indicating a luminous colour with a red-orange tint. The values of h* (hue) and C* (chroma) were 0.89 ± 0.0002 and 53.91 ± 0.10, respectively, indicating the colour quality and the intensity. These results differ from those of phenolic extracts containing anthocyanins obtained from fruits and vegetables since the colour depends on the source of anthocyanins (Bridle & Timberlake, 1997; Sari *et al*., 2012; Fernández-López *et al*., 2013). Fernández-López *et al*., (2013) reported values of 62.6, 31.5 and −18.8 for L*, a* and b*, respectively, for red cabbage extract, indicating a red-blue tint, differing from the sorghum extract.

Changes in colour and total anthocyanins in model beverages containing sulphite and ascorbic acid, under light exposure

Changes in total anthocyanin content

The variation of total anthocyanin content for the model beverage systems in the presence and absence of light is presented in Table 1. There was a significant difference between storage conditions (presence or absence of light) only for model beverages containing sulphite (E_{Su1} and E_{Su2}) on days 7, 14 and 21 (Table 1). For the other model beverages (E_S, E_{Ac1} and E_{Ac2}), there was no significant difference, showing that for both storage conditions, the anthocyanin content averages were equal at each time and for each type of beverage. Total anthocyanin content remained similar within the first 7 days of storage and showed a decrease from day 7 to day 14 for model beverages E_S, E_{Ac2} and E_{Ac2}, with an average of 22.93% and 22.82% for storage in the presence and absence of light, respectively. After day 14 for these three model beverages, the anthocyanin content remained stable in both storage conditions (Table 1).

Model beverages with addition of sulphite (E_{Su1} and E_{Su2}) showed an opposite behaviour, in which the total anthocyanin content increased during 21 days of storage in the presence and absence of light (Table 1). For the model beverage E_{Su1}, there was a significant increase in the anthocyanin content from day 0 to 7 in the presence (83.86%) and absence of light (51.45%); it remained stable in the absence of light until day 14 and then increased 69.11% compared to day 0. For

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Table 1 Variation of total anthocyanin content (mg LE/100 mL) in model beverages containing sulphite and ascorbic acid during 21 days of storage under light exposure.

		Days							
Model beverage systems	**Storage condition**	**0**		**7**		**14**		**21**	
E_S	Light	2.91 ± 0.13	$^{A,ab,\alpha}$	2.97 ± 0.08	$^{A,c,\alpha}$	2.24 ± 0.02	$^{A,c,\beta}$	2.20 ± 0.05	$^{A,c,\beta}$
	Dark	2.91 ± 0.13	$^{A,\mathbf{ab},\alpha}$	2.92 ± 0.03	$^{A,\mathbf{bc},\alpha}$	2.27 ± 0.09	$^{A,\mathbf{c},\beta}$	2.30 ± 0.07	$^{A,\mathbf{c},\beta}$
E_{Ac1}	Light	2.87 ± 0.08	$^{A,ab,\alpha}$	2.93 ± 0.03	$^{A,c,\alpha}$	2.25 ± 0.01	$^{A,c,\beta}$	2.20 ± 0.06	$^{A,c,\beta}$
	Dark	2.87 ± 0.08	$^{A,\mathbf{ab},\alpha}$	2.90 ± 0.02	$^{A,\mathbf{bc},\alpha}$	2.31 ± 0.07	$^{A,\mathbf{c},\beta}$	2.24 ± 0.11	$^{A,\mathbf{c},\beta}$
E_{Ac2}	Light	2.95 ± 0.14	$^{A,a,\alpha}$	2.94 ± 0.04	$^{A,c,\alpha}$	2.31 ± 0.01	$^{A,c,\beta}$	2.20 ± 0.01	$^{A,c,\beta}$
	Dark	2.95 ± 0.14	$^{A,\mathbf{a},\alpha}$	3.02 ± 0.03	$^{A,\mathbf{b},\alpha}$	2.24 ± 0.15	$^{A,\mathbf{c},\beta}$	2.36 ± 0.05	$^{A,\mathbf{c},\beta}$
E_{Su1}	Light	2.69 ± 0.03	$^{A,b,\gamma}$	4.95 ± 0.13	$^{A,a,\alpha}$	4.76 ± 0.05	$^{A,a,\alpha\beta}$	4.70 ± 0.10	$^{A,a,\beta}$
	Dark	2.69 ± 0.03	$^{A,\mathbf{b},\gamma}$	4.10 ± 0.05	$^{B,\mathbf{a},\beta}$	4.19 ± 0.33	$^{B,\mathbf{a},\beta}$	4.55 ± 0.09	$^{A,\mathbf{a},\alpha}$
E_{Su2}	Light	2.89 ± 0.09	$^{A,ab,\beta}$	3.42 ± 0.05	$^{A,b,\alpha}$	3.27 ± 0.08	$^{A,b,\alpha}$	3.45 ± 0.02	$^{A,b,\alpha}$
	Dark	2.89 ± 0.09	$^{A,\mathbf{ab},\beta}$	2.78 ± 0.06	$^{B,\mathbf{c},\beta}$	2.77 ± 0.22	$^{B,\mathbf{b},\beta}$	3.45 ± 0.10	$^{A,\mathbf{b},\alpha}$

Results were expressed as means ($n = 3$). Means followed by equal capital letters in the column for the same treatment within the same day do not differ in the storage condition; equal lowercase letters in bold in the column for different treatments within the same day do not differ in the storage in the dark; equal lowercase letters in the column for different treatments within the same day do not differ in the storage in the light; equal Greek letters on the line for the same treatment in the same storage condition do not differ between days by the Tukey test (5% of probability).
E_S, Extract without additives; E_{Ac1}, Extract added with ascorbic acid (0.015 g/100 mL); E_{Ac2}, Extract added with ascorbic acid (0.03 g/100 mL); E_{Su1}, Extract added with sulphite (0.002 g/100 mL); E_{Su2}, Extract added with sulphite (0.004 g/100 mL); LE, Luteolinidin equivalent.

the storage under light exposure, the E_{Su1} anthocyanin content reduced from day 14 until the last day of storage and the final content was 74.63% higher than day 0. There was no change in anthocyanin content of the model beverage E_{Su2} from day 0 to day 14 in the dark, with a significant increase until the last day of storage (Table 1). For the same model beverage (E_{Su2}), in the presence of light, there was a significant increase in the anthocyanin content between day 0 and 7 and it remained constant until day 21.

Sulphite is an additive used as a preservative for foods and beverages and its interaction with anthocyanins often leads to bleaching, which is generally reversible and results from a nucleophilic addition of sulphite on the C ring of the flavylium cation, leading to the formation of colourless sulphonates (Timberlake & Bridle, 1967; Cavalcanti *et al*., 2011). In our study, because of the immediate bleaching effect by sulphite addition, the concentration of sorghum phenolic extract used to reach absorbance between 0.8 and 0.9 was higher than the concentration used for the other treatments. Therefore, as a reversible reaction, the anthocyanin content was higher on the last day of storage compared to the first day. Our results corroborate the findings of Ojwang & Awika (2010) who observed a higher final absorbance for sorghum 3-deoxyanthocyanidins after 21 days in the presence of sulphite; however, they used an excess of sulphite (molar ratio ~1:40, pigment/sulphite).

The recovery of anthocyanins content for sulphite treatments was more evident in light (Table 1) and can be justified by the formation of sulphonate complexes that could possibly copigment with the flavylium cation (AH+), increasing colour intensity (Ojwang & Awika, 2010). In addition, the sorghum 3-deoxyanthocyanins tend to more readily self-associate over time compared to other anthocyanins, due to the presence of a hydrophobic pyrylium ring, protecting the AH+ from further nucleophilic attack (Ojwang & Awika, 2010). It was also observed that the recovery of anthocyanins for the E_{Su1} treatment was more evident than for the E_{Su2} treatment, in both storage conditions (Table 1). This fact may be related to the concentration of sulphite present in the E_{Su2} treatment, twice as high as the concentration found in the E_{Su1} treatment, disfavouring equilibrium of the flavylium cation over the colourless bisulphite adduct (Jurd, 1963).

Kinetics of anthocyanin degradation

The results of 3-deoxyanthocyanins degradation kinetics are presented in Table 2. Values of the degradation constant (k), half-life ($t_{1/2}$) and percentage of degradation of sorghum 3-deoxyanthocyanins in the presence of ascorbic acid (E_{Ac1} and E_{Ac2}) did not differ in the presence or absence of light. For the treatments containing sulphite (E_{Su1} and E_{Su2}), no degradation kinetics were reported, since the total anthocyanin content of the model beverages in the last day of storage (day 21) was higher than day 0 (i.e. there was no degradation) (Table 1).

The degradation percentages of total anthocyanins for the extract without additives (E_s) stored in the presence and in the absence of light were 24.16% and 20.72%, respectively (Table 2). There was no significant difference in the degradation of 3-

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Table 2 3-deoxyanthocyanins degradation kinetics in model beverages containing ascorbic acid for 21 days under light exposure

		Treatments			
	Condition	**E_S**	**E_{Ac1}**	**E_{Ac2}**	**Means**
k (1/day)($\times 10^{-3}$)	Light	0.55 ± 0.13	0.54 ± 0.04	0.58 ± 0.09	0.56 ± 0.02^{A}
	Dark	0.46 ± 0.14	0.49 ± 0.04	0.44 ± 0.08	0.46 ± 0.02^{A}
	Means	0.51 ± 0.06^{a}	0.52 ± 0.04^{a}	0.51 ± 0.10^{a}	
$t_{1/2}$ (days)	Light	54.70 ± 14.67	53.22 ± 3.97	50.38 ± 7.44	52.76 ± 2.20^{A}
	Dark	66.29 ± 20.85	59.68 ± 5.64	66.94 ± 12.18	64.30 ± 4.03^{A}
	Means	60.50 ± 8.19^{a}	56.44 ± 4.57^{a}	58.66 ± 11.73^{a}	
%degradation	Light	24.16 ± 5.13	23.99 ± 1.50	25.38 ± 3.38	24.51 ± 0.76^{A}
	Dark	20.72 ± 5.43	21.74 ± 1.75	19.87 ± 3.05	20.77 ± 0.94^{A}
	Means	22.44 ± 2.43^{a}	22.86 ± 1.60^{a}	22.63 ± 3.90^{a}	

Results were expressed as means ± standard deviation ($n = 3$). Means followed by equal lowercase letters in the lines and uppercase letters in the column do not differ at 5% probability by the Tukey test. There was no significant interaction.
E_S, Extract without additives; E_{Ac1}, Extract added with ascorbic acid (0.015 g/100 mL); E_{Ac2}, Extract added with ascorbic acid (0.03 g/100 mL).

deoxyanthocyanins between the storage conditions, indicating the stability of the sorghum anthocyanins to the fluorescent light corresponding to storage light. The light exposition of anthocyanins leads to photodegradation of flavylium cations into colourless forms (i.e. the carbinol pseudobase and chalcone) (Dyrby *et al*., 2001).

The lack of substitution at the C-3 position in the 3-deoxyanthocyanins results in a region between C-5 and C-4 that has greater hydrophobicity than its anthocyanin analogues and is less reactive with hydrophilic molecules. This region makes 3-deoxyanthocyanins less susceptible against to nucleophilic attack and hydration, which are mechanisms for the structural transformation of anthocyanins in solution to be transformed in colourless forms (Awika, 2008; Yang *et al*., 2014). Awika (2008) reported good stability of pure 3-deoxyanthocyanidins stored under fluorescent laboratory light at 25 °C for 135 days. The colour retention was 57% and 73% for the 5,7-dimethoxylated luteolinidin and apigeninidin, respectively, indicating that the methoxylation on the A-ring significantly improves the stability of 3-deoxyanthocyanins, and that methoxylation at position 5, and not at position 7, was the key to the improved stability. In our study, since a crude phenolic extract was used, the high stability to light could also be related to the copigmentation and complexation of 3-deoxyanthocyanins with other phenolic compounds present in the extract, as reported by Awika *et al*., (2004).

The stability of sorghum 3-deoxyanthocyanins to light is advantageous, considering that common anthocyanins (i.e. cyanidin and delphinidin glycosides) can lose more than 90% of the initial concentration after 15 days under fluorescent light (Baublis *et al*., 1994). Eiro & Heinonen (2002) reported that pure malvidin 3-glucoside lost its colour after 55 days at room temperature in daylight. In a recent study, Chen *et al*., (2018) reported that red cabbage anthocyanin monomers decreased up to 81% after 24 h under natural indoor light. Considering our study, these results highlight the greater light stability of 3-deoxyanthocyanins compared to other anthocyanins which implies that sorghum food colourant can be applied to foods and non-food products commonly exposed to fluorescent light.

Regarding the effect of the ascorbic acid, the degradation percentages for the treatments E_{Ac1} and E_{Ac2} in the presence of light were 23.99% and 25.38%, respectively, not differing from each other. This indicates that the variation in the ascorbic acid concentration (0.015% or 0.03%) did not interfere in the degradation of sorghum 3-deoxyanthocyanins (Table 2). The same behaviour was observed for treatments E_{Ac1} and E_{Ac2} stored in the dark, with values of 21.74% and 19.87%, respectively. The kinetics (k and $t_{1/2}$ values) and the percentage of degradation for the treatments E_{Ac1} and E_{Ac2} under both storage conditions (light or dark) did not differ from the treatment E_S (control), which demonstrates that sorghum 3-deoxyanthocyanins were very stable under light exposure and the presence of ascorbic acid, in concentrations commonly found in Brazilian beverages.

Brenes *et al*., (2005) reported that in a grape juice model system, the concentration of anthocyanins decreased 82% in the presence of ascorbic acid during 20 days in the dark at 25 °C. However, in our study, the 3-deoxyanthocyanins showed stability under ascorbic acid which can also be related to their different structure: the lack of –OH group in the C-3 position. Furthermore, crude sorghum extract contains other more stable forms of 3-deoxyanthocyanins (glycosides, acyl glycosides, etc.) and it is also likely to contain

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other phenolic compounds that could act as copigments, enhancing the anthocyanin stability in the presence of ascorbate degradation products (Ojwang & Awika, 2008). Our results corroborate earlier findings of Ojwang & Awika (2008) who reported that, in the presence of 500 mg L^{-1} of ascorbic acid, red cabbage pigment lost 85% of its colour while sorghum extract lost only 31% of its colour after 21 days at pH 2.0 under fluorescent laboratory lighting. The concentration of ascorbic acid used by these authors was superior to those used in our study, which can explain why our anthocyanin stability was superior with degradation of 23.99% for E_{Ac1} and 25.38% for E_{Ac2} under light exposure for 21 days.

Changes in colour of model beverages containing sorghum anthocyanins

Variations of the chroma (C *) and hue (h*) coordinates in the model beverages are presented in Table S1. All treatments were found to be within the first quadrant (MacDougall, 2010) with positive values of a* and b*, that is, between the red and yellow colours, respectively, with plenty of light (high value of L*); high C* values, indicating that the treatments had pure and intense colours; and low h* values, indicating a shade that tends to red, relating to the anthocyanin pigment present in the sorghum bran.

Chroma values (C*) decreased over 21 days, for all treatments in both storage conditions, with E_{Su1} being the only exception in which the value on day 0 did not differ from day 21 (Table S1). This fact could be related to the recovery of anthocyanins which was more evident for the E_{Su1} model beverage. When analysing each treatment separately in the two storage conditions, it was found that the decrease in saturation was more pronounced in the presence of light. These results indicate that there was a decrease in the intensity of the red/orange colour (decrease in C* values) as a consequence of the loss of anthocyanins and formation of degradation compounds such as colourless pseudobase or carbinol (Cevallos-Casals & Cisneros-Zevallos, 2004). Regarding the presence of food additives, there was no significant difference in the C* values among the treatments E_S, E_{Ac1} and E_{Ac2} on days 7 and 21. Moreover, treatments containing sulphite (E_{Su1} and E_{Su2}) presented different C* values between them, and among the other treatments. The decrease in saturation (C* value) was less pronounced for treatments containing sulphite (E_{Su1} and E_{Su2}), since the addition of sulphite leads to bleaching, which is reversible, then there is less change in the intensity of colour in these treatments.

The hue values (h*) decreased from day 7, between the storage conditions for all treatments (Table S1). This fact indicates the change in the shade of the original colour. There was no significant difference in the h* parameter among the E_S, E_{Ac1} and E_{Ac2} model beverages in both storage conditions during 21 days, which is related to the fact that there was no significant difference in the kinetics of 3-deoxyanthocyanins degradation for these treatments. There was a decrease in h* parameter for the treatments E_S, E_{Ac1}, E_{Ac2} from day 14 and for E_{Su1} and E_{Su2} from day 7 in the storage in dark and the lowest values were reported on day 21. For all treatments in the light, there was a decrease in h* from day 7 with the lowest values also on day 21. The decrease in the h* and C* values was more accentuated in all treatments in the presence of light, indicating the action of fluorescent light on the stability of anthocyanins (Sari *et al*., 2012).

The global colour variation (ΔE*) is presented in Fig. 1. The parameter ΔE* is calculated from the changes in the coordinates L*, a* and b* between the beginning and the end of the storage period. For all treatments, ΔE* was greater than 1, in all storage conditions. This indicates that there was a noticeable difference in the colour of the beverages between the beginning and the end of the experiment, since an ΔE* of 1.0 represents the threshold of perception of colour difference to the human eye (Gonnet, 1998). However, small colour differences are not sufficient to mischaracterise a product or negatively influence its acceptance. Changes in h*, C* and ΔE* are in accordance with

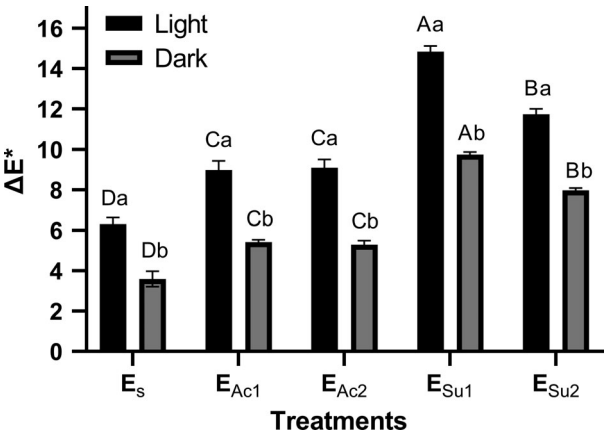

Figure 1 Global colour variation in model beverages containing sulphite and ascorbic acid for 21 days under light exposure. Results were expressed as means and error bars indicate ± standard deviation. Bars bearing different lowercase letters (a,b) mean significant differences between storage conditions (light/dark), at a 5% probability by the Tukey test. Bars bearing different uppercase letters (A–D) mean significant differences among treatments (additives) at 5% probability by the Tukey test. E_S, Extract without additives; E_{Ac1}, Extract added with ascorbic acid (0.015g/100mL); E_{Ac2}, Extract added with ascorbic acid (0.03g/100mL); E_{Su1}, Extract added with sulphite (0.002g/100mL); E_{Su2}, Extract added with sulphite (0.004g/100mL)

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results reported on model beverages coloured with Andes Berry anthocyanin powder after storage for 71 days at 16°C in daylight (Estupiñan *et al*., 2011).

The E_S treatment (control) showed the lowest overall colour variation (ΔE*) in both storage conditions, with values of 6.29 and 3.59 in the presence and absence of light, respectively (Fig. 1). The highest values of ΔE*were found in the treatments E_{Su1} and E_{Su2} in the presence of light, with values of 14.84 and 11.74, respectively. In the absence of light, the ΔE* values were 9.74 and 7.97, respectively. Moreover, for sulphite treatments, there was a decrease in the values of the L* axis and the b* axis, indicating lower luminosity and less yellowish tint; and an increase in the a* axis indicating a greater red tint (data not shown).

Changes in colour and total anthocyanins in model beverages subjected to heat treatment

Changes in total anthocyanin content

The total anthocyanin content of model beverages before and after heat treatment at 80 °C (0, 5 and 25 min) is presented in Table 3. The total anthocyanin content did not differ ($P > 0.05$) for the same treatment over time and among different treatments over time.

Sorghum 3-deoxyanthocyanins showed greater heat stability in the presence of additives. The 3-deoxyanthocyanins resist fragmentation during thermal treatment, they have greater deprotonation rate constant than hydration rate constant, hence convert less to the colourless carbinol pseudobases in aqueous solutions, which would lead to less formation of chalcones after heat treatment (Yang *et al*., 2014). According to Yang *et al*., (2014), 3-deoxyanthocyanidins were remarkably stable under the severe heat treatment of 121 °C/30 min, with the highest thermal stability at pH 1 and 2, with higher than 80% colour retention and 68%–78% colour retention at pH 3–6. In the same study, the crude extract retained 89% of colour after the 95 °C/2 h heat treatment.

For sulphite treatments (E_{Su1} and E_{Su2}), unlike their behaviour during the stability study under light for 21 days of storage, there was no increase in the content of total anthocyanins during the heat treatment (Table 3). The bleaching reaction by the sulphite is reversible over time and, since it was used for only 5 and 25 min in the heat stability study, there was no recovery of the anthocyanin content.

Table 3 Total anthocyanins content of model beverages containing ascorbic acid and sulphite after heat treatment at 80 °C for 0, 5 and 25 min

	Total anthocyanins (mg LE/100 mL)			
Treatments	**0 min**	**5 min**	**25 min**	**Means**
E_S	2.69 ± 0.02	2.60 ± 0.05	2.66 ± 0.06	2.65 ± 0.04^A
E_{Ac1}	2.60 ± 0.05	2.63 ± 0.03	2.63 ± 0.03	2.62 ± 0.01^A
E_{Ac2}	2.62 ± 0.01	2.66 ± 0.10	2.74 ± 0.03	2.67 ± 0.06^A
E_{Su1}	2.66 ± 0.08	2.67 ± 0.06	2.65 ± 0.09	2.66 ± 0.01^A
E_{Su2}	2.58 ± 0.06	2.61 ± 0.02	2.62 ± 0.01	2.60 ± 0.02^A
Means	2.63 ± 0.04^a	2.64 ± 0.03^a	2.66 ± 0.05^a	

Results were expressed as means ± standard deviation. Means followed by equal lowercase letters in the rows and uppercase letters in the columns do not differ at 5% probability by the Tukey test. There was no significant interaction.

E_S, Extract without additives; E_{Ac1}, Extract added with ascorbic acid (0.015 g/100 mL); E_{Ac2}, Extract added with ascorbic acid (0.03 g/100 mL); E_{Su1}, Extract added with sulphite (0.002 g/100 mL); E_{Su2}, Extract added with sulphite (0.004 g/100 mL). LE, Luteolinidin equivalent.

Changes in colour of model beverages containing sorghum anthocyanins

Colour stability of sorghum anthocyanins after heat treatment was demonstrated by colorimetric analysis (Tables S2 and S3). The stability of sorghum anthocyanins after heat treatment can also be related to colour stability. Regarding chroma (C*), there was no significant difference for each treatment before and after heat treatment for 25 min (Table S2). Moreover, the hue (h*) did not differ during the heat treatment for 5 and 25 min for the same treatment and among different treatments (Table S3), indicating that there was no change in shade of the model beverage systems. Such results demonstrate once again the colour stability of sorghum anthocyanins to heat treatment (Yang *et al*., 2014; Akogou *et al*., 2018).

Global colour variation after heat treatment is presented in Table S3. There was a significant difference between ΔE* after 5 and 25 min of heat treatment, with values of 0.89 and 1.98, respectively. However, despite the global variation in colour being greater after heat treatment for 25 min, the values were very close to 1.0, indicating little variation perceptible to the human eye (Gonnet, 1998).

Conclusions

Crude 3-deoxyanthocyanin extract added in model beverage systems at pH 3.5 showed high stability during storage for 21 days under fluorescent light exposure, presence of ascorbic acid (0.015% and 0.03%) and sulphite (0.002% and 0.004%) in concentrations commonly found in Brazilian beverages and heat treatment (80 °C for 5 or 25 min). Overall, colour parameters (h*, C* and ΔE*) of the model beverages were stable during storage under light and heat treatment. Therefore, the crude phenolic extract obtained from sorghum bran, containing

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3-deoxyanthocyanins, has potential to be utilised as natural food colourant and nutraceutical ingredient in many beverage products.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

Mariana Barbosa: Formal analysis (equal); Investigation (equal); Methodology (equal); Writing-original draft (equal). **Thaís Rigolon:** Data curation (equal); Methodology (equal). **Larissa Borges:** Data curation (equal); Methodology (equal). **Valeria Queiroz:** Data curation (equal); Methodology (equal); Resources (equal); Writing-review & editing (equal). **Paulo Cesar Stringheta:** Funding acquisition (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal). **Frederico Barros:** Conceptualization (lead); Funding acquisition (equal); Methodology (equal); Project administration (lead); Resources (equal); Supervision (lead); Writing-review & editing (equal).

Ethics approval

Ethics approval was not required for this research.

Data availability statement

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Experimental design under light exposure or in the absence of light: 5 treatments (E_s, E_{Su1}, E_{Su2}, E_{Ac1} and E_{Ac2}), in 3 repetitions with 4 beverage bottles in each repetition (B1, B2, B3, B4).

Figure S2 Experimental design in the absence of light in a water bath at 80°C: 5 treatments (E_s, E_{Su1}, E_{Su2}, E_{Ac1} and E_{Ac2}), in 3 repetitions with 3 beverage bottles in each repetition (B1, B2, B3).

Table S1 Variation of Chroma (C*) and hue (h*) coordinates in model beverages containing sulfite and ascorbic acid for 21 days under light exposure.

Table S2 Variation of Chroma (C*) coordinate in model beverages containing sulfite and ascorbic acid after heat treatment at 80 °C (0, 5 and 25 min).

Table S3 Global color variation (ΔE*) and variation of hue (h*) in model beverages containing sulfite and ascorbic acid after heat treatment at 80 °C (0, 5 and 25 min).

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