**MARIANA PEREIRA BARBOSA**

# EFFECT OF LIGHT, FOOD ADDITIVES AND HEAT ON THE STABILITY OF SORGHUM 3-DEOXYANTHOCYANINS IN MODEL BEVERAGES

Dissertation presented to the Universidade Federal de Viçosa as part of the requirements of the Graduate Program in Food Science and Technology to obtain the title of *Magister Scientiae.*

Advisor: Frederico Augusto Ribeiro de Barros
Co-advisor: Paulo César Stringheta

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Assent:

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Advisor

*To my parents and my sister,*
*For the strength to keep me going,*
*For all the moments of pure happiness,*
*For unconditional love. My wonderwall.*
*Paulo, Edmeia and Maria Eduarda,*
*I dedicate.*

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## ABSTRACT

BARBOSA, Mariana Pereira, M. Sc., Universidade Federal de Viçosa, September, 2020. **Effect of light, food additives and heat on the stability of sorghum 3-deoxyanthocyanins in model beverages.** Advisor: Frederico Augusto Ribeiro de Barros. Co-advisor: Paulo César Stringheta.

Sorghum has high levels of 3-deoxyanthocyanins (DXA), which is known to possess unique chemical and bioactive properties. The DXA do not have the hydroxyl group at the C-3 position, which improves their stability compared to anthocyanins from fruits and vegetables. However, crude sorghum DXA stability in model beverages under fluorescent light exposure is unknown. The aim of this work was to evaluate the stability of sorghum DXA in model beverages (pH 3.5) elaborated with sorghum bran phenolic extract, containing ascorbic acid and sulfite, 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, in two different concentrations, during storage under light exposure (23.99-25.38%) and absence of light (19.87-21.74%). These values are much lower compared to literature for anthocyanins under similar conditions. The addition of sulfite caused an initial bleaching reaction, but as reversible reaction, the anthocyanin content was higher on the last day of storage compared to the first day. Moreover, it was observed that there were no significant differences in total anthocyanin content of all treatments subjected to the heat treatment (80°C for 5 and 25 min). Overall, color parameters (h*, C* and ΔE*) of the model beverages were stable during storage under light and heat treatment. In conclusion, crude sorghum DXA are very stable to fluorescent light exposure and heat, thus have great potential to be used as natural food colorants and bioactive ingredients by the food industry.



Keywords: Natural colorants. Bioactive compounds. Sulfite. Ascorbic acid. Anthocyanins. *Sorghum bicolor* L.

## RESUMO

BARBOSA, Mariana Pereira, M.Sc., Universidade Federal de Viçosa, setembro de 2020. **Effect of light, food additives and heat on the stability of sorghum 3-deoxyanthocyanins in model beverages.** Orientador: Frederico Augusto Ribeiro de Barros. Coorientador: Paulo César Stringheta.

O sorgo tem altos níveis de 3-desoxiantocianinas (DXA), que são conhecidas por possuir propriedades químicas e bioativas únicas. As DXA não possuem o grupo hidroxila na posição C-3, o que melhora sua estabilidade em relação às antocianinas de frutas e vegetais. No entanto, a estabilidade das DXA do extrato fenólico bruto de sorgo em bebidas modelo sob exposição à luz fluorescente é desconhecida. O objetivo deste trabalho foi avaliar a estabilidade das DXA de sorgo em bebidas modelo (pH 3,5) elaboradas com extrato fenólico de farelo de sorgo, contendo ácido ascórbico e sulfito, sob exposição à luz fluorescente e submetidas a tratamento térmico. Não houve diferença significativa na degradação das DXA durante o armazenamento sob exposição à luz (24,16%) e ausência de luz (20,72%). A degradação das DXA não diferiu na presença de ácido ascórbico, em duas concentrações diferentes, durante o armazenamento sob exposição à luz (23,99-25,38%) e ausência de luz (19,87-21,74%). Esses valores são muito mais baixos em comparação com a literatura para antocianinas em condições semelhantes. A adição de sulfito causou uma reação inicial de branqueamento, mas como reação reversível, o teor de antocianinas foi maior no último dia de armazenamento em relação ao primeiro dia. Além disso, observou-se que não houve diferenças significativas no teor de antocianinas totais de todos os tratamentos submetidos ao tratamento térmico (80 °C por 5 e 25 min). No geral, os parâmetros de cor (h*, C* e ΔE*) das bebidas modelo permaneceram estáveis durante o armazenamento sob luz e tratamento térmico. Em conclusão, as DXA do extrato fenólico bruto do sorgo são muito estáveis à exposição à luz fluorescente e ao calor, portanto, têm grande potencial para serem utilizadas como corante natural de alimentos e ingredientes bioativos pela indústria de alimentos.



Palavras-chave: corantes naturais. Compostos biativos. Sulfito. Ácido ascórbico. Antocianinas. *Sorghum bicolor* L.

**TABLE OF CONTENTS**

# 1 INTRODUCTION

Anthocyanins are flavonoids and make up the largest group of water-soluble pigments found in the plant kingdom, ranging from red to blue in many fruits and vegetables (Bridle & Timberlake, 1997). These pigments have been extensively studied due to their bioactive properties and potential for application as natural food colorants (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).

Despite their use as natural food colorants, anthocyanins still have some limitations, since they are susceptible to degradation by factors such as light, pH, heat and enzymes (Francis & Markakis, 1989). The presence of food additives also affects the stability of anthocyanins. Sulfites used in the food industry produce discoloration in anthocyanins (Cavalcanti et al., 2011). The bleaching reaction is generally reversible depending on the structure of the pigments involved, pH and oxygen availability in 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 pigments in the presence of oxygen (Sondheimer & Kertesz, 1953). Thus, several researches 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).

Sorghum DXA, unlike anthocyanins, do not have the hydroxyl group at the C-3 position (Dykes & Rooney, 2006). This characteristic improves its stability compared to anthocyanins from fruits and vegetables (Awika et al., 2004a). Sorghum DXA showed high color retention than anthocyanins when processing at 95°C/2h and 121°C/30 min, and at pH variations (Akogou et al., 2018; Yang et al., 2014). Ojwang & Awika (2010) demonstrated that, even after the bleaching effect of the sorghum DXA due to the addition of sulfite, they recovered partially or totally their color within 14 and 21 days, at pH 3.0 and 1.8. Moreover, even in the presence of ascorbic acid, DXA showed low color 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; Luo et al. 2018; Suganyadevi et al. 2013), which make them potential bioactive food ingredients.

However, there have been no studies on the stability of crude sorghum DXA in model beverage systems to 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 sulfite, under fluorescent light exposure and subjected to heat treatment.

# 2 LITERATURE REVIEW

## 2.1 Color

The color attribute is generally considered to be one of the most important in the appearance of any food. Almost all food products have an acceptable color range, which depends on a wide range of factors, including the variability between consumers, their age and ethnic origin and the physical nature of the environment at the time of the judgment (Francis & Markakis, 1989).

Appearance is used throughout the production-storage-use-marketing chain as the main means of judging the quality of individual units of products, being evaluated considering their size, shape, color and the absence of visual defects (Kays, 1999). Therefore, color is an important sensory attribute to provide basic quality information for human perception and has a close association with quality factors such as freshness, maturity, variety and convenience, and food safety and, therefore, is a classification factor for most food products (McCaig, 2002).

First, the consumer judges a food by its color and then evaluates the other sensory attributes, such as taste and aroma. In this way, the food industry strives to measure and control the color of its products through effective color inspection systems to measure such information quickly and objectively during processing operations and storage periods (Wu & Sun, 2013).

Traditional instruments, such as colorimeters and spectrophotometers, have been used in the food industry for color measurement (Wu & Sun, 2013). Under the specified lighting environment, these instruments provide a quantitative measure, simulating the way in which the human eye sees the color of an object (McCaig, 2002). The human eye distinguishes colors according to the sensitivity of different conical cells of the retina to light under different wavelengths. There are three types of photoreceptor cells (cones) with peak sensitivity at short (bluish tones, 420 and 440 nm), medium (greenish tones, 530 and 540 nm) and long (red tones, 560 and 580 nm) (Wu & Sun, 2013).

The CIE system, created in 1931, uses the X (red), Y (green) and Z (blue) coordinates, through transmittance by spectrophotometric measurements that are later mathematically transformed into color coordinates. Such values specify the amount of colors to describe a color in the visible light spectrum (Macdougall, 2010).

Spectrophotometers with an extended spectral range that includes the visible region are also widely used for color measurement throughout the food and agricultural industry (McCaig, 2002). Such devices generate the spectral distribution of the sample's transmittance or reflectance. XYZ values are calculated, depending on the illuminant, the measurement geometry and the observer (Korifi et al., 2013).

Colorimeters are used to measure the color of primary radiation sources that emit light and secondary radiation sources that reflect or transmit external light (Macdougall, 2010). Its measurement is quick and simple. Using the space CIE L *, a *, b *, known as CIELAB, measurements are made regarding the luminosity coordinates (L *) and visually uniform chromaticity coordinates (a * and b *) (Figure 1). The values of L *, a * and b * are proportional to the XYZ values of the CIE system (Korifi et al., 2013).

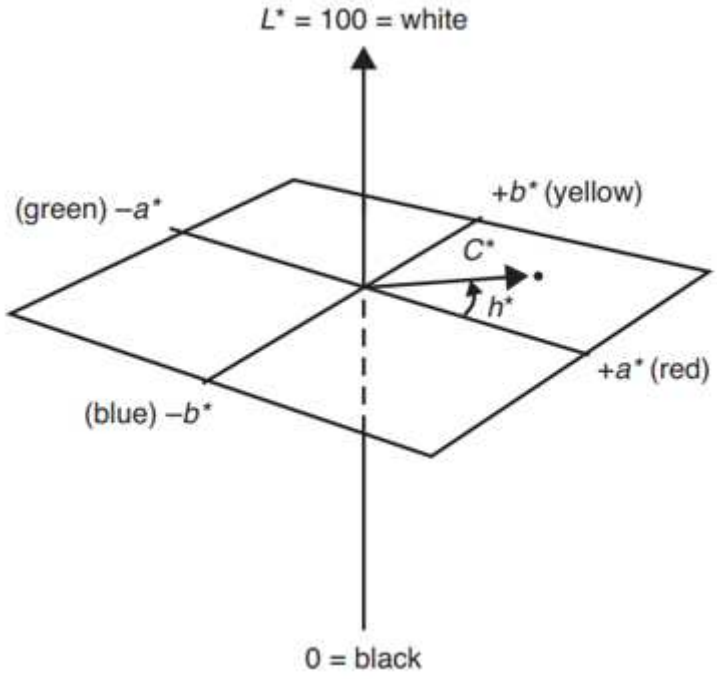



**Figure 1:** CIELAB uniform diagram showing the red / green (a* +/-) and yellow / blue (b* +/) opponent, coordinates for luminosity (L*), chroma (C*) and angle of hue (h*) (Macdougall, 2010)

The coordinate L*, referring to luminosity, varies from zero, representing the least luminous tone (black) to 100, representing the most luminous tone (colorless). The axes a* and b* correspond to chromatic coordinates that vary from negative to positive, where a* varies from green tones (-80) to red tones (+100) and b* varies from blue tones (-50) and yellow (+70) (Korifi et al., 2013). The hue (h*) characterizes the color quality as red, green and blue, for example, being associated with a certain wavelength of the visible spectrum (Equation 1). The chroma or saturation (C*) represents the intensity or quantity of a hue, allowing the distinction between strong and weak colors (Equation 2). In the CIELAB system, the total color difference (ΔE*) can be calculated using the luminosity coordinate and visually uniform chromaticity coordinates (Equation 3).

$$h^* = \arctan(\frac{b^*}{a^*}) \quad \text{(Eq. 1)}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \text{(Eq. 2)}$$

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

## 2.2 Natural food colorants

Color and appearance are determining factors for the acceptance of a product by the consumer, resulting in the use of food colorants by the food industry. The main reasons for using these additives are the compensation of color loss due to exposure to light, air, temperature and storage conditions, the intensification of the natural colors of the food in order to make it even more attractive, the supply of color for those colorless foods and, finally, make foods easier to identify (Amchova et al., 2015).

In general, food colorants can be classified into insoluble and soluble substances, which are subdivided into natural, semi-synthetic and synthetic. Natural ones are those obtained from foods or other natural sources, such as riboflavin, chlorophylls, carotenes, betalain and anthocyanins, presenting less stability to processing (temperature and pH variations, for example) than synthetic food colorants (Amchova et al., 2015). Under Brazilian law, according to RDC n°4 of November 24$^{th}$, 1988 of the Conselho Nacional de Saúde (BRASIL, 1988), permitted food colorants are divided into four groups, with the respective labeling codes: natural food colorantss (CI ), artificial (C.II.), synthetic identical to natural (C.III.) and caramel (CV).

Among the natural food colorants, betalain, cochineal, carotenoids and anthocyanins stand out. Betalains exhibit colors ranging from red-violet (betacyanins) to yellow-orange (betaxanthines), with only beet pigment used commercially in dairy products, meat and many

others. The cochineal pigment is extracted from the insect *Dactylopius coccus* Costa, presenting a red color and being widely used in gelatines, bakery products, dairy products and drinks (Carocho et al., 2015). Carotenoids and anthocyanins are among the most used in the food industry. Carotenoids are fat-soluble, capable of yellow to red coloring in food products, obtained mainly from carrots, tomatoes and peppers. On the other hand, anthocyanins are soluble in water, having as commercial sources grapes and red cabbage. These natural food colorants have been used in soft drinks, confectionery and fruit preparations (Carocho et al., 2015; Castañeda-Ovando et al., 2009).

Replacing synthetic food colorants with natural ones is challenging since natural food colorants are generally less stable, more expensive, require more material to achieve a color intensity equivalent to synthetic, and have a limited range of colorimetric hues (Rodriguez-Amaya, 2019). However, synthetic food colorants have been linked to toxic effects, which can lead to allergies and cancer risks (El-Wahab & Moram, 2013). Another important point is that natural food colorants, in addition to providing color, can act as antioxidants and preservatives (Carocho et al., 2015). Therefore, the use of natural food colorants is a current commercial trend due to consumers' concern with the safety of synthetic food colorants, reinforced by the possible health benefits of consuming products containing natural antioxidants (Amchova et al., 2015; Rodriguez-Amaya, 2019).

## 2.3 Anthocyanins

Anthocyanins are flavonoids and make up the largest group of water-soluble pigments found in the plant kingdom, ranging from red to blue in many fruits and vegetables (Bridle & Timberlake, 1997). These pigments have been extensively studied in these foods due to their bioactive properties and potential for application as natural food colorants (Castañeda-Ovando et al., 2009).

Anthocyanins are anthocyanidin polyhydroxy and polymethoxy glycosides derived from the 2-phenylbenzopyrilic cation (flavilium cation) (Mazza & Brouillard, 1990). Thus, anthocyanidins are the basic structures of anthocyanins, consisting of an aromatic ring [A] attached to a heterocyclic ring [C] that contains oxygen, which is also linked by a carbon-carbon bond to a third aromatic ring [B] (Figure 2). When anthocyanidins are found in their glycosidic form (linked to a portion of sugar), they are known as anthocyanins (Castañeda-Ovando et al., 2009).

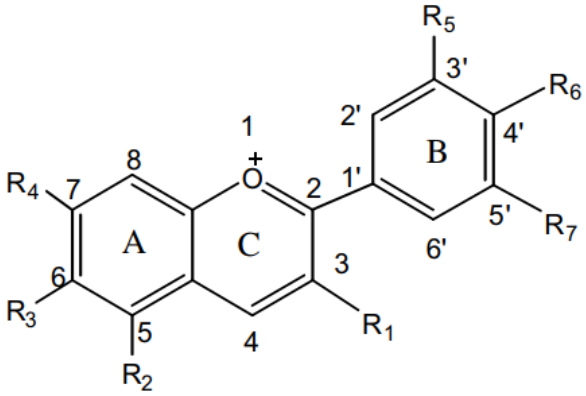



**Figure 2:** General structure of anthocyanidins (Castañeda-Ovando et al., 2009)

There is a huge variety of anthocyanins in the nature. The main differences between them are the number and position of hydroxyl groups, the degree of methylation of hydroxyl groups, the nature, number and position of sugars linked to their structure and the nature and number of aliphatic or aromatic acids attached to sugar. The six most commonly found anthocyanins are pelargonidin, cyanidin, peonidin, delfinidin, petunidin and malvinidin, which differ in the number of hydroxyl and methoxy groups in ring B of the flavilium cation (Table 1). The distribution of the six most common anthocyanidins in fruits and vegetables is: 50% cyanidin, 12% delfinidin, 12% pelargonidin, 12% peonidin, 7% petunidin and 7% malvinidin. The most common glycosidic derivatives in nature are 3-monosides, 3-biosides, 3.5 and 3.7 diglycosides, with cyanidin-3-glycoside being the most common anthocyanin (Clifford, 2000; Kong et al., 2003).

**Table 1:** Main anthocyanidins found in nature

| Anthocyanidin | Substitution pattern | | | | | | | Color |
|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | |
| Pelargonidin | OH | OH | H | OH | H | OH | H | Orange/salmon |
| Cyanindin | OH | OH | H | OH | OH | OH | H | Magenta/crimson |
| Peonidin | OH | OH | H | OH | $OCH_3$ | OH | H | Magenta |
| Delphinidin | OH | OH | H | OH | OH | OH | OH | Purple/blue |
| Petunidin | OH | OH | H | OH | $OCH_3$ | OH | OH | Purple |
| Malvidin | OH | OH | H | OH | $OCH_3$ | OH | $OCH_3$ | Purple |

(Cavalcanti et al., 2011)

Anthocyanins are the most studied natural pigments for application as a natural food colorant (Carocho et al., 2015) Although there are approximately 400 types of anthocyanins present in various plants such as grapes, cherry, strawberries, blackberries, red cabbage and

sweet potatoes, few present themselves as a commercial source of this pigment. Only grapes and red cabbage are used commercially (Carocho et al., 2015; Kong et al., 2003).

Despite their use as natural food colorants, anthocyanins still have some limitations, due to their instability, being susceptible to degradation by factors such as light, pH, heat, sulfite, ascorbic acid, enzymes, among others (Francis & Markakis, 1989). Thus, the research has been directed towards the search for better sources and improvement of the extraction efficiency and stability (Cavalcanti et al., 2011; Rodriguez-Amaya, 2019).

### *2.3.1 Bioactive properties*

In addition to their application as a food colorant, anthocyanins also have desirable functional attributes. Such attributes arouse the interest of the food industry in developing products rich or enriched with natural antioxidants, adding naturalness and convenience, in addition to sensory acceptance (Amchova et al., 2015; Carocho et al., 2015).

Anthocyanins have been shown to have high antioxidant activity and, therefore, can be used to inhibit oxidation reactions (Diaconeasa et al., 2015). In addition, these pigments have other bioactive properties such as anti-inflammatory (Wang & Mazza, 2002) and chemopreventive activities (Zhao et al., 2004). Anthocyanins also aid in the modulation of arterial protection (Colantuoni et al., 1991), inhibition of platelet aggregation (Morazzoni, P.; Magistretti, 1990) and endothelial protection (Youdim et al., 2002).

More recent studies also demonstrate 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).

### *2.3.2 Stability*

Anthocyanins are highly unstable and easily susceptible to degradation by several factors (Francis & Markakis, 1989). Among these, pH stands out, since, due to the ionic nature of anthocyanins, changes in the structure of the molecule can occur according to the predominant pH, determining in different colors and hues at different pH values (Mazza & Brouillard, 1990). When in acidic solutions, anthocinins are presented as four main species in equilibrium: quinoid base (QB), flavilium cation (FC), carbinol or pseudobase (PB) and chalcone (CH) (Figure 3) (Cavalcanti et al., 2011).

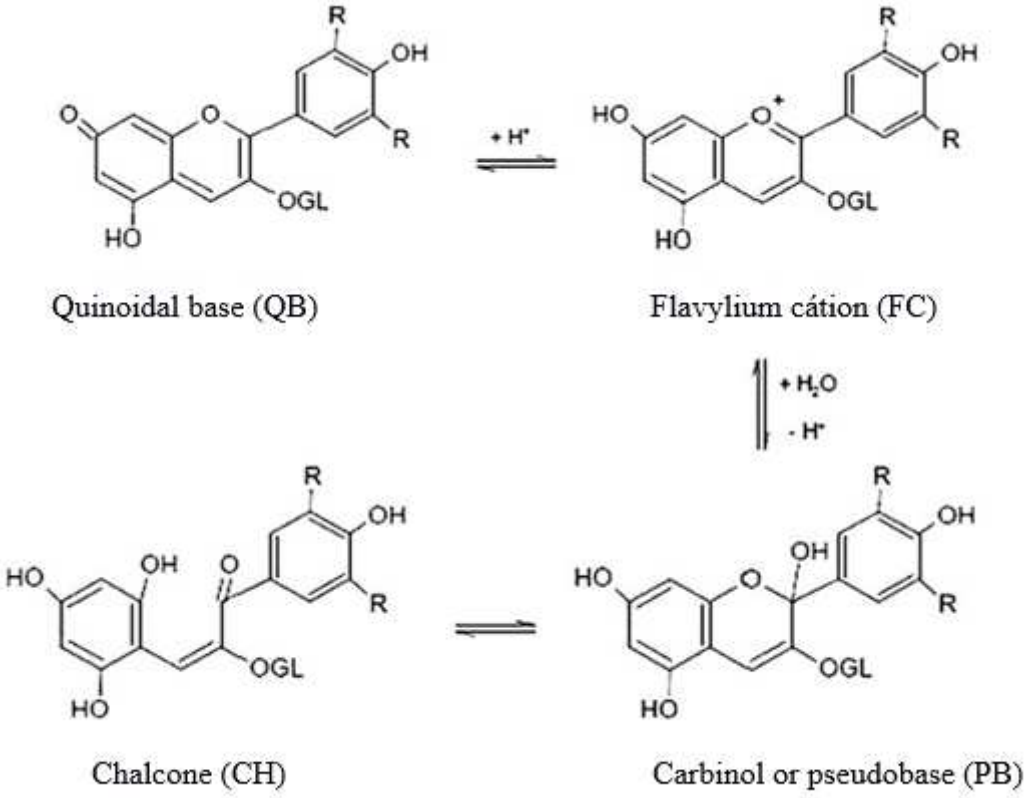



**Figure 3:** Main equilibrium anthocyanin species: the quinoidal base (QB), the flavylium cation (FC), the carbinol or pseudobase (PB) and the chalcone (CH) (Cavalcanti et al., 2011)

Under acidic conditions (pH <2), anthocyanin exists mainly in the form of flavilium cation (FC), with a red color. The increase in pH values leads to rapid loss of the proton, producing forms of quinonoidal base (QB) with blue or violet coloaration. At the same time, the hydration of the flavilium cation (FC) occurs, generating carbinol or pseudobase (PB), which slowly reaches equilibrium with the colorless chalcone (CH). The relative amounts of forms of the four forms in the equilibrium condition vary according to the pH (Iacobucci & Sweeny, 1983).

Another factor that affects the stability of anthocinins is co-pigmentation. Some studies suggest that the co-pigmentation of anthocyanins with other compounds (co-pigments) is the main mechanism of color stabilization in plants (Davies & Mazza, 1993; Mazza & Brouillard, 1990). The interaction between anthocyanin and co-pigment can be performed in different ways, depending on the species in interaction (Figure 4). If the co-pigment is another anthocyanin, a self-association or intramolecular co-pigmentation is formed (Figures 4A and 4B); when the interaction is with a metal, a metal complexation is performed (Figure 4C); in

the case of co-pigments with free electron pairs, intermolecular copigmentation occurs (Figure 4D) (Castañeda-Ovando et al., 2009).

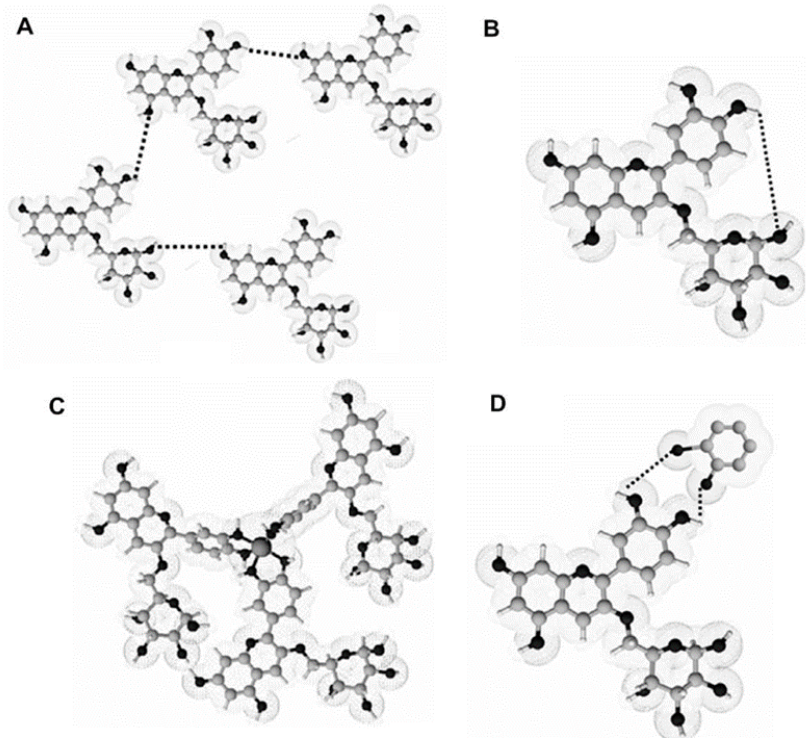



**Figure 4:** Anthocyanins: (A) self-association; (B) intramolecular copigmentation; (C) metal complexation; (D) intermolecular copigmentation (Castañeda-Ovando et al., 2009)

Regarding co-pigmentation with phenolic compounds, the resulting total co-pigmentation is based on two effects. The first effect, leads to the formation of the π- π complex that causes changes in the spectral properties of the molecules in the flavilium ion, increasing the absorption intensity (hyperchromic effect) and its wavelength (bathochromic shift). In the second effect, the flavilium form is stabilized by the π complex, which shifts the balance in such a way that the red color increases (Figure 5). Thus, the magnitude of the co-pigmentation effect is dependent on pH, since at low pH values, all anthocyanin molecules are in the form of flavilium, and at high pH values, anthocyanin is in its form of carbinol pseudobase, which is colorless (Castañeda-Ovando et al., 2009).

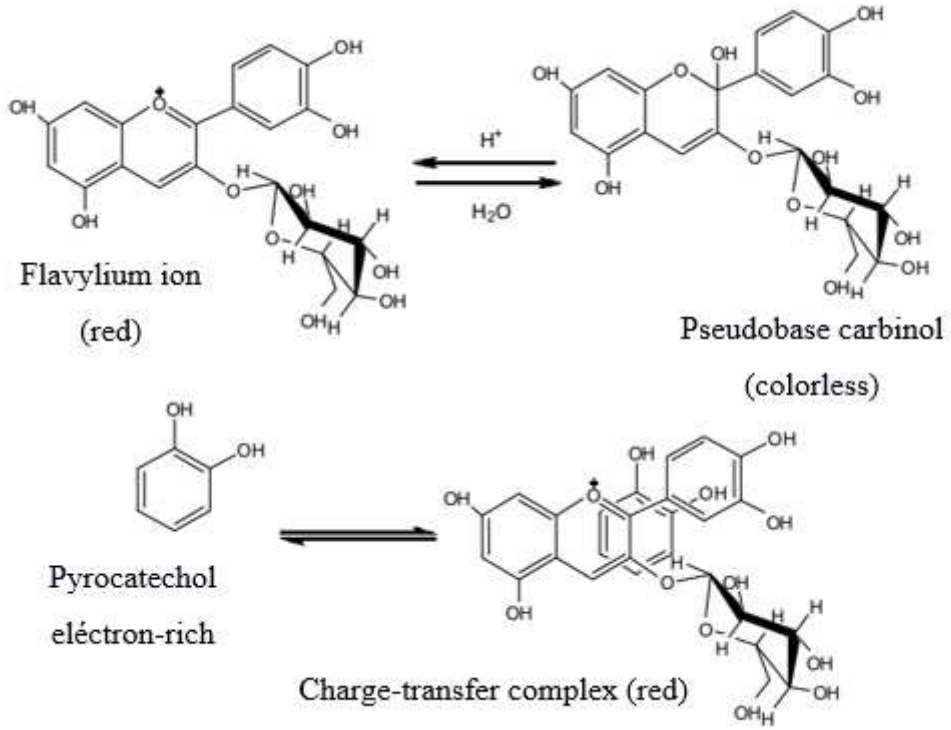



**Figure 5:** Example of anthocyanins stabilisation via formation of the complex by charge transfer (interaction of the anthocyanin with a phenolic compound) (Castañeda-Ovando et al., 2009)

Heat and oxygen are also important factors related to anthocyanin degradation. The stability of anthocyanins and all pigments found in food decreases during processing and storage as the temperature increases (Patras et al., 2010). The increase of the temperature in the values of pH 2-4 induces the loss of the glycosylic portions of the anthocyanins, by hydrolysis of the glycosidic bond (Adams, 1973). This leads to an additional loss of anthocyanin color, since aglyclones are much less stable than their glycosidic forms. It is postulated that the formation of a chalcone is the first step in the thermal degradation of anthocyanins. In addition, thermal degradation can lead to the formation of brown products, especially in the presence of oxygen (Adams, 1973; Markakis, 1982). Its presence accelerates the anthocyanins degradation process. The influence of oxygen can occur through a direct oxidative mechanism and / or through the action of oxidizing enzymes. The polyphenoloxidase enzyme (PPO) catalyzes the oxidation of chlorogen acid to its corresponding quinone which reacts with anthocinins, forming brown colored condensation products (Patras et al., 2010). In addition, oxygen-induced anthocinins instability is affected by pH; the higher the pH, the greater the degradation of this pigment in the presence of oxygen (Markakis, 1982).

Light is a factor that can affect anthocinins in two different ways, being essential for the biosynthesis of these pigments, but also accelerating their degradation (Markakis, 1982). Kearsley & Rodriguez (1981) found that anthocyanins (water-soluble anthocyanin powder) had their color preserved better when stored in the absence of light, in which the difference was observed after 24 hours when compared to anthocyanins stored under incidence of light. Results obtained by Stringheta (1991) indicate that the destructive effect of light is present in the anthocyanins present in *Melinis minutiflora*, with considerable intensity, in which such degradation is accelerated when there is an association of the factors light and oxygen.

The presence of additives also affects the stability of anthocyanins. The effect of sugar, for example, depends on the structure of anthocyanin, concentration and type of sugar. Sugars, as well as their breakdown products, are known to decrease anthocyanin stability (Cavalcanti et al., 2011). However, sucrose has been linked to the protection of anthocyanins, preventing browning and formation of polymeric pigments, probably due to the inhibition of enzymatic reactions or the prevention of different condensation reactions by sucrose (Wrolstad et al., 1990).

Sulfites used in the food industry produce discoloration in anthocyanins (Cavalcanti et al., 2011). The bleaching reaction is generally reversible depending on the structure of the pigments involved, pH and oxygen availability in solution (Timberlake & Bridle, 1967). Sulphites dissociate almost instantly in solution in three species, depending on the thermodynamic constant and the pH of the solution. Such species are sulfur dioxide ($SO_2$), sulfite ion ($SO_3^{2-}$) and bisulfite ion ($HSO_3^-$). The bleaching effect of sulfites on anthocyanins in solution results from the nucleophilic addition of these molecular species to the C-ring of the flavilium cation, leading to the formation of colorless sulfonates (Jurd, 1963).

Ascorbic acid is one of the most common food additives used, especially in beverages, for nutritional purposes or as a preservative (Ojwang & Awika, 2008). However, its use in products containing anthocinins is limited since it significantly accelerates the destruction of these pigments in the presence of oxygen (Sondheimer & Kertesz, 1953). Some factors can affect such degradation, such as the presence of antioxidant copigments and temperature (Brenes et al., 2005). The mechanism proposed by Jurd (1963) and later reiterated by Poei-Langston & Wrolstad (1981) for the degradation of anthocyanins in the presence of ascorbic acid consists of the direct condensation of ascorbic acid on carbon 4 of the anthocyanin molecule. In other mechanisms, the anthocyanin color loss caused by ascorbic acid may occur due to oxidative cleavage of the pyrilium ring by a free radical mechanism, in which ascorbic acid acts as a molecular oxygen activator, producing free radicals (Iacobucci & Sweeny, 1983).

## 2.4 Sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the main cereal crops grown in the world, reaching a production of more than 57 million tons in 2017. The United States has the largest world production, followed by Nigeria (FAO, 2017). This grain is recognized as a versatile and drought-tolerant crop, well adapted to superior productivity in a warmer climate and increasing water scarcity (Girard & Awika, 2018). Despite these agronomic attributes, its use in the world is limited in relation to its potential, being mostly used for animal feed, being part of the food culture of the population of several countries in Asia and Africa (Dykes et al., 2009).

However, there is growing interest in the use of sorghum in human consumption since this grain is unique among the main cereals, having high levels of a diverse range of bioactive compounds not common to other cereals, with the potential to promote benefits to human health (Girard & Awika, 2018). Among these, there are benefits to the intestinal microbiota and parameters related to obesity, oxidative stress, inflammation, diabetes, dyslipidemia, cancer and hypertension (de Morais Cardoso et al., 2015).

According to the Companhia Nacional de Abastecimento (CONAB), a public company linked to the Ministry of Agriculture (MAPA) in Brazil, the area planted with sorghum in the 2018/19 harvest corresponded to 785.9 thousand hectares. In addition, grain productivity in this same harvest reached 1.9 million tons, with the states of Goiás and Minas Gerais being the largest producers (CONAB, 2018) (Figure 6).

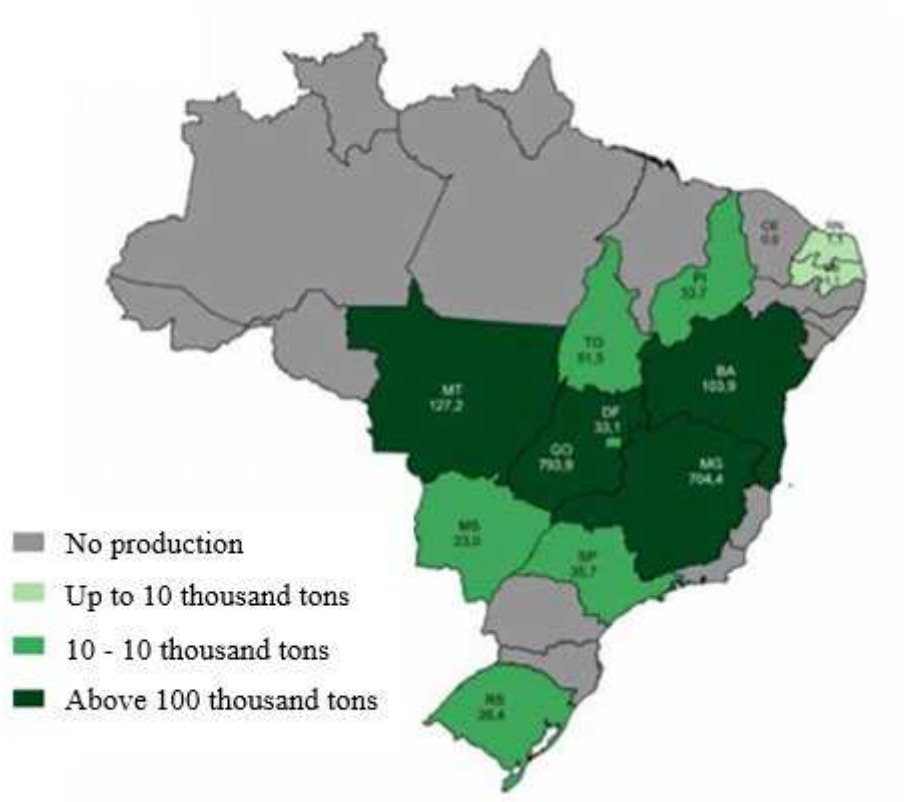


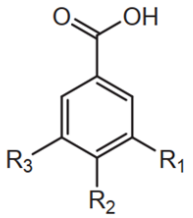

**Figure 6:** Map of agricultural sorghum production in Brazil (CONAB, 2018)

Currently, due to the modern lifestyle and the high rates of overweight and obesity, consumers have been looking for more nutritious products, with functional properties and potential health benefits. As previously reported, sorghum has a wide range of bioactive compounds, making it an excellent choice for the development of these new products (Queiroz et al., 2018). In Brazil, the consumption of sorghum is still incipient, although some products, such as cereal bars, cookies, pasta and breakfast cereals have already been developed with the sorghum grain, showing good acceptance (Anunciação et al., 2017; Queiroz et al., 2012). A recent study by Queiroz et al (2018) obtained sensory acceptance of a nutritious and low-calorie powder mixture for drinks, with extruded sorghum flours. do Prado et al (2019) carried out another application of sorghum with the elaboration of bovine hamburgers added with sorghum flour to replace the isolated soy protein, in which there were higher scores for flavor, texture, general acceptability and purchase intent compared to the traditional formulation of humburger.

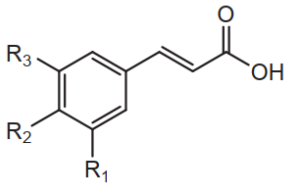
In sorghum, as in other cereal grains, bioactive compounds are located mainly in the bran fraction (pericarp, testa and aleurone), coexisting with the abundant cell wall polysaccharides. The diversity of polyphenols is responsible for a wide variety of pericarp colors in sorghum, ranging from cream white to red, lemon yellow and black, and therefore can

also provide a natural means to diversify the sensory visual appeal of food (Girard & Awika, 2018). The color and thickness of the pericarp, presence of pigmented testa and color of the endosperm affect the coloring characteristic of the sorghum grains (Dykes et al., 2009). The main phenolic compounds in sorghum are phenolic acids, flavonoids (eg anthocyanins and flavanols) and condensed tannins.

Phenolic acids (Figure 7), classified as benzoic and cinnamic, are found in all sorghum genotypes, located in the pericarp, testa, aleurone and endosperm of the grain. As with other cereal grains, 70-95% of sorghum phenolic acids are covalently linked to cell wall polysaccharides, commonly referred to as bound phenolic acids (Awika et al., 2018). Ferulic acid derivatives represent the most abundant group, corresponding to about 90% of the bound phenolic acids in sorghum (Girard & Awika, 2018).




Major **benzoic acids** in sorghum;
Gallic acid, $R_1 = R_2 = R_3 = OH$
Protocatechuic acid, $R_1 = R_2 = OH$, $R_3 = H$
Vanillic acid, $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$
p-Hydroxybenzoic acid, $R_1 = R_3 = H$, $R_2 = OH$



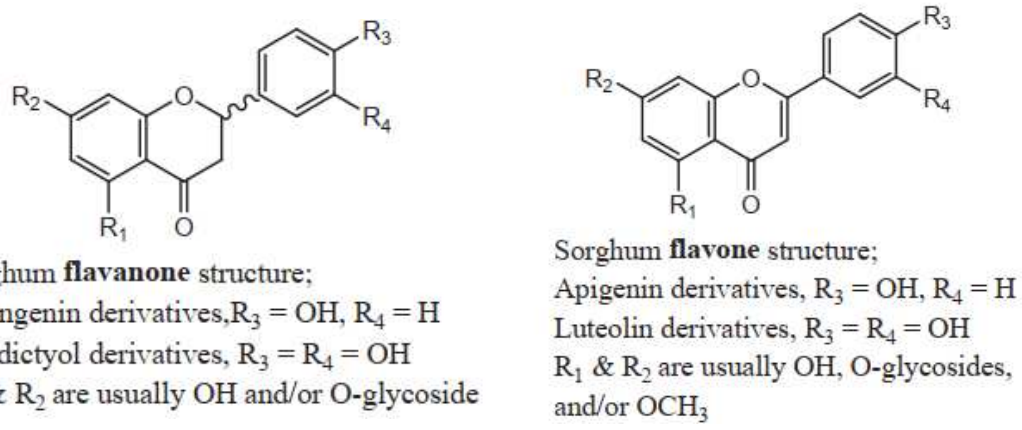

Major **cinnamic acids** in sorghum;
Ferulic acid, $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$
Caffeic acid, $R_1 = R_2 = OH$, $R_3 = H$
Sinapinic acid, $R_1 = R_3 = OCH_3$, $R_2 = OH$
p-Coumaric acid, $R_1 = R_3 = H$, $R_2 = OH$

**Figure 7:** Structure of the main phenolic acids in sorghum (Girard & Awika, 2018)

Unlike other cereals, sorghum has significant levels of monomeric and/or polymeric flavonoids, the main ones being 3-deoxyanthocyanins, flavones and flavanones (Dykes et al., 2009). The vast majority of monomeric sorghum flavonoids have a phenol or catechol group in ring B, thus being derived from naringenin (apigenin and apigeninidin and proapigeninidin derivatives) or eriodicthiol (luteolin or luteolinidin, proluteolinidin and procyanidin derivatives) (Girard & Awika, 2018). The main structural difference between flavones and flavanones is that flavones have a double bond C2 = C3, so they are conjugated and relatively flat, whereas flavanones are chiral in C2 due to the saturated structure C2-C3 (Awika et al., 2018) (Figure 8).

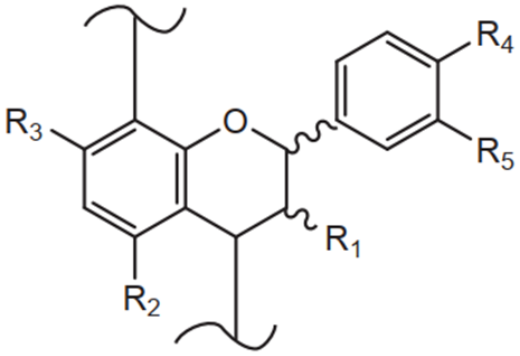
Another important factor is that the levels of flavones in sorghum (20–390 μg/g) are similar to wheat (190–365 μg/g), the major grain with the highest levels of these compounds, being mainly O-glycosides or aglycone, while other cereal grains contain mainly C-glycosides

(Awika et al., 2018; Dykes et al., 2011; Hernández et al., 2011; Yang et al., 2015). Unlike flavones, flavanones are generally rare in cereal grains, at least in significant amounts (Girard & Awika, 2018). Sorghum appears to be an exception with one of the highest levels of flavanones (up to 1800 µg/g) identified in specific phenotypes with lemon yellow color pericarp (Dykes et al., 2011; Yang et al., 2015). However, even in sorghum, flavanones are found only in specific phenotypes, indicating a strong genetic component in their accumulation (Awika et al., 2018).




.**Figure 8:** Sorghum flavonoids (Girard & Awika, 2018)

The condensed tannins stand out in the composition of the sorghum grains (Figure 9). These phenolic compounds also known as proanthocyanidins are high molecular weight polyphenols, polymers of flavan-3-ol, which have a high antioxidant capacity and are found on the testa of the grain, a structure located between the pericarp and the endosperm of the grain. Only varieties with a pigmented testa have condensed tannins (Dykes & Rooney, 2006). In addition to tannins, many sorghum flavonoids were isolated and identified, such as anthocyanins, which is the main class of flavonoids studied in these grains (Dykes & Rooney, 2006).




Sorghum **proanthocyanidin** unit structure;
Proapigeninidin , $R_1 = R_5 = H$, $R_4 = OH$
Proluteolinidin, $R_1 = H$, $R_4 = R_5 = OH$
Procyanidin, $R_1 = R_4 = R_5 = OH$
$R_1$ & $R_2$ are usually OH, but can rarely have $OCH_3$

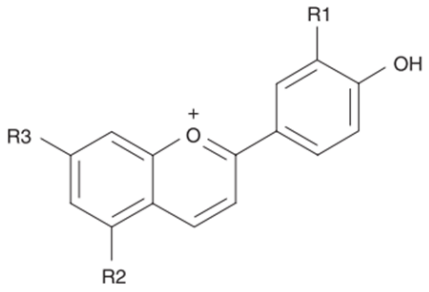
**Figure 9:** Structure of sorghum proanthocyanidins (Girard & Awika, 2018)

## 2.5 Sorghum anthocyanins

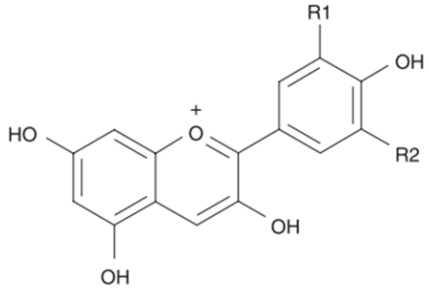
There are limited data on the types and levels of anthocyanins in cereals, probably because they are not considered a commercially significant source. However, due to the demand for natural sources of food colorants, alternative sources of anthocyanins are becoming increasingly important, such as sorghum (Awika et al., 2004a).

Despite the availability of natural anthocyanin food colorants from other sources, sorghum grains have a great advantage related to ease of handling compared to sources of anthocyanins from fruits and vegetables. Sorghum tissue is non-succulent and is typically harvested in low humidity, so drying and long-term storage is possible at low cost. Fruits and vegetables, on the other hand, have high humidity and enzymatic activity, which results in higher storage and processing costs. In addition, the levels of pigments derived from sorghum currently compete favorably with the anthocyanin content in commercial pigment sources (Petti et al., 2014) Another important commercial factor is that the sorghum anthocinins are concentrated in the bran, which is, to a large extent, a residue of the grain processing (grinding, alcohol production). Thus, the additional value added by its valuation would significantly increase the competitiveness of sorghum (Girard & Awika, 2018).

In nature, the six common anthocyanidins are cyanidin, delfinidin, malvinidin, pelargonidin, petunidin and peonidin (Clifford, 2000). However, the main anthocyanins in sorghum are 3-deoxyanthocyanins, which comprise luteolidin and apiginidin, including their methoxylated derivatives, 5-methoxyluteolinidin and 7-methoxypigeninidin (Awika & Rooney, 2004; Dykes & Rooney, 2006). Such anthocinins are different from the common ones since they do not present the hydroxyl group at the C-3 position (Figure 10), presenting low distribution in nature (Clifford, 2000; Dykes & Rooney, 2006).




Apigeninidin: R1 = H; R2 = OH; R3 = OH
Apigeninidin 5-glucoside: R1 = H; R2 = OGlc; R3 = OH
5-Methoxyapigeninidin: R1 = H; R2 = $OCH_3$; R3 = OH
7-Methoxyapigeninidin: R1 = H; R2 = OH; R3 = $OCH_3$
7-Methoxyapigeninidin 5-glucoside: R1 = H; R2 = OGlc; R3 = $OCH_3$
Luteolinidin: R1 = OH; R2 = OH; R3 = OH
Luteolinidin 5-glucoside: R1 = OH; R2 = OGlc; R3 = OH
5-Methoxyluteolinidin: R1 = OH; R2 = $OCH_3$; R3 = OH
5-Methoxyluteolinidin 7-glucoside: R1 = OH; R2 = $OCH_3$; R3 = OGlc
7-Methoxyluteolinidin: R1 = OH; R2 = OH; R3 = $OCH_3$




Pelargonidin: R1 = H; R2 = H
Cyanidin: R1 = OH; R2 = H
Delphinidin: R1 = OH; R2 = OH
Peonidin: R1 = $OCH_3$; R2 = H
Petunidin: R1 = $OCH_3$; R2 = OH
Malvidin: R1 = $OCH_3$; R2 = $OCH_3$

**Figure 10:** Structures of 3-deoxyanthocyanidins and their derivatives found in sorghum compared to the six common anthocyanins found in fruits, vegetables and some cereals (Dykes & Rooney, 2006)

Nip & Burns (1971, 1969) were able to isolate and identify apigeninidin, apigeninidin-5-glycoside, luteolinidin and luteolinidin-5-glycoside in varieties of red and white sorghum, using paper chromatography. Cyanidin and pelargodine have also been identified (Yasumatsu et al., 1965). In addition, Gous (1989) reported luteolinidin and apigeninidin as the main anthocyanidins in a variety of black sorghum.

Sorghum with a black pericarp have the highest levels of 3-deoxyanthocyanins (Awika et al., 2004a, 2004b; Gous, 1989) which are concentrated in the bran (Awika et al., 2005). Using the differential pH method of Fuleki & Francis (1968), Awika et al (2004b) reported that the anthocyanin content of a black sorghum bran was 3 to 4 times greater than that of whole grain and had at least twice the level of anthocyanins (10.1 mg/g) compared to sorghum bran. red

(3.6 mg/g) and brown (3.6 mg/g). In addition, Awika et al (2004a, 2004b) reported that luteolinidin and apigeninidin represented 36-50% of the total anthocyanin content in black sorghum (Tx430 Black) and brown (Hi Tannin) brans. The levels of anthocyanin in sorghum compare to that of fruits and vegetables commonly used as commercial sources of anthocyanin, in addition to other cereals (Table 2).

**Table 2:** Sorghum bran anthocyanin content compared to fruits, vegetables and cereals

| Commodities | Content (mg/g) | Main anthocyanins |
|---|---|---|
| Black sorghum bran | 4.0-9.8 | Apigenidin e luteolinidin |
| Brown sorghum bran | 1.6-3.9 | Apigenidin e luteolinidin |
| Red sorghum bran | 3.3 | Apigenidin |
| Blueberry | 0.2-5.0 | Malvinidin e delfinidin |
| Red grapes | 0.3-7.5 | Peonidin, malvinidin e petunidin |
| Strawberry | 0.2-0.9 | Pelargonidin |
| Red cabbage | 0.3-0.9 | Cianidin |
| Purple maize | 1.6 | Cianidin |
| Black rice | 0-4.9 | Cianidin e peonidin |

(Awika & Rooney, 2004)

Due to the structural characteristic of 3-deoxyanthocyanins, that is, the absence of hydroxyl at the C-3 position, they present chemical and biochemical properties very different from common anthocyanins (Ojwang & Awika, 2008). Such characteristic is related to improve the stability of sorghum anthocyanins compared to fruits and vegetables conventionally used as a commercial source of this pigment (Awika et al., 2004a). The lack of substitution at C-3 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 to nucleophilic attack and hydration, which is a primary mechanism for the structural transformation of anthocyanins in solution to colorless forms (Awika, 2008; Yang et al., 2014).

Regarding the stability to pH variation, 3-deoxyanthocyanins are a particular class of anthocyanins since the constant deprotonation of their flavilium cation is greater than hydration. Consequently, a solution of 3-deoxyanthocyanins remains colored at high pH (Akogou et al., 2018). The colored species (cation flavilium and quinoidal bases) typically predominate at any pH value, whereas common anthocyanins are mostly converted to colorless pseudobase carbinol and chalcones at pH> 3 (Mazza & Brouillard, 1987). A study by Akogou et al (2018)

evaluated the effects of processing and storage on the stability of sorghum apigenidin natural colorant, in which the aqueous extracts of the bran showed good stability at heat treatments and at pH 6-10.

The 3-deoxyanthocyanins also present stability to food additives. Ascorbic acid, for example, is one of the most common additives, used mainly in beverages, but its application in products containing anthocyanin as natural colorant is limited by the fact that it significantly accelerates the color loss of the pigment in the presence of oxygen (Ojwang & Awika, 2008). However, a study by (Ojwang & Awika, 2008), demonstrated that the crude extract of sorghum bran is very stable in the presence of ascorbic acid (500 mg/L) with 31% loss of color at pH 2 and increases of 1.9 and 1.3 times in color intensity at pH 3.2 and 5.0, respectively, highlighting the greater stability of sorghum anthocyanins. Sulfite is also a food additive that, when interacting with anthocyanins, causes its color loss (Jurd, 1963). Ojwang & Awika (2010) reported greater resistance to bleaching apigeninidin than its methoxylated derivatives in the presence of sulfite. However, all pigments recovered partially or totally the color within 14 and 21 days, at pH 3.0 and 1.8, indicating a favorable balance to the flavilium cation.

Yang et al (2014) reported that both the crude extract of sorghum 3-deoxyanthocyanins and such pure anthocyanins showed good thermal stability after heat treatments at 95°C/2h and 121°C/30 min. The extract tended to be less stable than pure anthocinins, suggesting that other phenolic constituents present could affect the thermal stability of the pigments. However, a study by Awika (2008) found that the presence of phenolic acids could improve the color stability of sorghum anthocyanins, especially ferulic and tannic acid. In addition, evidence points to greater stability of the crude extract of sorghum bran at pH variations compared to the pure 3-deoxyanthocyanins (Awika et al., 2004b). This fact was also observed in relation to the stability to ascorbic acid in which, due to the presence of other phenolics, the crude extract proved to be more stable since these compounds can act as copigments (Ojwang & Awika, 2008).

Due to its better overall stability to food processing compared to the most common anthocyanins, there is considerable interest in using 3-deoxyanthocyanins as natural food colorant (Akogou et al., 2018). However, no studies were found in the literature related to the stability of sorghum anthocyanins to light exposure. It is known that, for conventional anthocyanins, light leads to the photodegradation of flavilium cations into colorless forms (Dyrby et al., 2001).

In addition to these advantages, 3-deoxyanthocyanins also have chemopreventive properties that make them potential bioactive food ingredients. Yang et al (2009) reported that

3-deoxyanthocyanins induce the activity of quinone reductase, a protective phase II enzyme, and inhibit the growth of esophageal cells and human colon cancer. Such anthocyanins have also been proven to be cytotoxic against human colon cancer cells (Shih et al., 2007). A recent study by Luo et al (2018) demonstrated that the anthocyanin extract of red sorghum bran, obtained by ultrasound, inhibited 88.52% of HepG2 cells (human hepatocarcinoma cells) at a concentration of 200 μg/mL. In addition, Suganyadevi et al (2013) found that 3-deoxyanthocyanins isolated from red sorghum bran showed 84.09% inhibition of human breast cancer cell proliferation (MCF 7) *in vitro*.

The growing demand for natural ingredients by consumers has forced large food companies to seek natural food colorants, presenting a renewed opportunity to explore the highly functional pigments of sorghum. Although the potential of sorghum 3-deoxyanthocyanins is recognized, such compounds are not yet commercially inserted, which may be related to the availability of low-cost synthetic food colorants and other natural sources of anthocyanins (Girard & Awika, 2018).

# 3 OBJECTIVES

## 3.1 General objective

To evaluate the stability of sorghum 3-deoxyanthocyanins in model beverages containing ascorbic acid and sulfite, under fluorescent light exposure and subjected to heat treatment.

## 3.2 Specific objectives

- To characterize the phenolic extract obtained from sorghum bran (total anthocyanins, total phenolic compounds, antioxidant capacity and colorimetry);
- To evaluate the effect of additives (sulfite and ascorbic acid) and fluorescent light exposure on the stability of sorghum anthocyanins present in model beverages, at room temperature;
- To evaluate the thermal stability of sorghum anthocyanins in model beverages containing sulfite and ascorbic acid.

# 4 METHODOLOGY

This study was carried out in the Laboratory of Pigments and Bioactive Compounds of the Department of Food Technology at Federal University of Viçosa, Minas Gerais.

## 4.1. Materials

Hydrochloric acid was purchased from Synth, sodium carbonate from Alphatec, potassium persulfate from Exôdo and the other reagents were purchased from Sigma Aldrich: Folin-Ciocalteau, gallic acid, sodium carbonate, Trolox, DPPH, ABTS, ascorbic acid and metabisulfite of sodium.

The sorghum genotype SC 084 (Figure 11A), harvested in 2018, was supplied by Embrapa Milho e Sorgo, from Sete Lagoas, Minas Gerais, Brazil. This genotype had the highest total anthocyanin content among 230 genotypes of a panel with high genetic variability evaluated by Emprapa Milho e Sorgo (Embrapa's private database, not published). Sorghum grains were kept under refrigeration (5°C ± 1) after harvest until use.

To obtain the sorghum bran (Figure 11B), 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 standardized on a 500 µm sieve. Sorghum bran was stored in plastic bags under refrigeration (5°C ±1).


**Figure 11:** Sorghum obtained from Embrapa Milho e Sorgo: (A) Sorghum grains and (B) Sorghum bran

B

## 4.2 Obtention and characterization of sorghum bran phenolic extract

The extraction was carried out following the ratio of 1:100 of bran: solvent using 70% v/v acidified ethanol/water (pH 2), under agitation (KLINE shaker, New Technique) at room temperature for 2 hours. The resulting extract was centrifuged at 5000 rpm 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 a volumetric flask. Subsequently, it was stored in amber bottles under refrigeration (5 °C ± 1). Characterization of the phenolic extract (total anthocyanins and phenolics, antioxidant capacity and colorimetric analysis) was done immediately after extraction.

### *4.2.1 Total anthocyanins*

Total anthocyanins was determined only at pH 1 (Awika et al., 2004b). The wavelength used was 480 nm. The calculation of the total anthocyanin content per 100 mL of extract was performed according to Equation 4. 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)/100mL of extract.

$$AntT = \frac{Abs\ x\ MM\ xFD\ x100}{\varepsilon} \qquad \text{(Eq. 4)}$$

Where AntT refers to the total anthocyanin content, Abs represents the absorbance, FD is the dilution factor, MM refers to the molar mass of luteolinidin (270g/mol) and ε represents the molar extinction coefficient.

### *4.2.2 Total phenolics*

The total phenolic content was determined by the method proposed by Singleton & Rossi (1965). This is a Folin-Ciocalteau spectrophotometric method, based on the reduction of phosphomolybdic-phosphotungstic acid by phenolic hydroxyls. The absorbance of the sorghum bran 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).

*4.2.3 Antioxidant capacity*

The antioxidant capacity of phenolic extracts was obtained using the ABTS and DPPH methods, described below.

*4.2.3.1 ABTS Method*

The determination of the antioxidant capacity by the TEAC assay (antioxidant capacity equivalent to the Trolox) was carried out according to the methodology proposed by Re et al (1999) using the ABTS radical (2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonated). Trolox (2-carboxylic acid-6-hydroxy-2,5,7,8 tetramethylchroman) was used as standard. Therefore, a standard curve was constructed at concentrations of 0-200 µmol/L. The reaction was carried out by homogenizing 0.5 mL of each concentration of the antioxidant and 3.5 mL of the radical solution in test tubes, kept away from light until the reaction stabilized.

To construct the curve of the phenolic extract samples, five sequential dilutions were performed, in which each 0.5 mL of extract was added to 3.5 mL of the ABTS radical solution. The reading was performed on a spectrophotometer (UV-1601 PC, Shimadzu) at 734 nm, after 6 min of reaction. Through the absorbance values, a sample graph was constructed, relating the concentration (mL of extract/L) versus absorbance. To determine the antioxidant capacity, absorbance equivalent to 100 µmol/L was obtained from the trolox standard curve equation. The value found was substituted in the equation of the curve of each sample, in order to obtain the extract volume (mL) equivalent to 100 µmol/L. This value was corrected in µmol trolox equivalent (TE) per liter of extract (µmol TE/L).

4.2.3.2 *DPPH Method*

The antioxidant capacity by the DPPH method was performed according to the methodology proposed by Kim et al (2002) with modifications. For the DPPH (2,2-diphenyl-1-picryl hydrazine) assay, a 0.1 mmol/L solution of DPPH was prepared in 80% ethanol. A standard curve was constructed using the trolox reagent. For samples, a curve was constructed with five sequential dilutions, with 0.5 mL of the extract being added to 3.5 mL of DPPH solution. After an hour of reaction, the reading was made on a spectrophotometer (UV-1601 PC, Shimadzu) at 517 nm. Through the absorbance values, a graph was constructed for the samples, relating the concentration (mL of extract/L) versus absorbance.

The determination of the antioxidant capacity was obtained through the absorbance equivalent to 125 µmol/L of the standard trolox curve equation. The value found was substituted in the equation of the sample curve line, in order to obtain the extract volume (mL) equivalent to 125 µmol/L. This value was corrected in µmol trolox equivalent (TE) per liter of extract (µmol TE/L).

*4.2.4 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 readed, using a 15 mL cuvette. The calculation of the parameters h* (hue) and c* (saturation) was performed, according to equations 5 and 6, respectively.

$$h^* = \arctan(\frac{b^*}{a^*}) \quad \text{(Eq. 5)}$$

$$c^* = \sqrt{a^{*2} + b^{*2}} \quad \text{(Eq. 6)}$$

## 4.3 Stability study

The stability study was performed in two steps, described below (sections 4.3.1 and 4.3.2). Model beverage systems were prepared using the sorghum bran phenolic extract in the presence of additives (sulfite 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 analyzed on days 0, 7, 14 and 21. For the heat stability, the analyzes were performed at 80 °C, at times 0, 5 min and 25 min.

*4.3.1 Stability of sorghum anthocyanins in model beverages containing sulfite and ascorbic acid, under light exposure*

Sorghum bran 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 metabisulfite ($E_{Su1}$); (3) extract + 0.004 g/100 mL of

sodium metabisulfite ($E_{Su2}$); (4) extract + 0.015 g/100 mL of ascorbic acid ($E_{Ac1}$); (5) extract + 0.03 g/100 mL of ascorbic acid ($E_{Ac2}$). Concentrations of sodium metabisulfite (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 authorized 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) (Figure 12).

Treatments were stored in transparent glass bottles, hermetically closed, with the use of nitrogen gas, under incidence of 40W fluorescent light, 2,500 lux, corresponding to storage light, in Tru-Vue 2 equipment (Datacolor), under room temperature (25 °C ± 1) (Figure 13). As control, equal treatments to the previous ones 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.

### *4.3.2 Stability of sorghum anthocyanins in model beverages containing sulfite and ascorbic acid subjected to heat treatment*

The different treatments obtained in the section 4.3.1 ($E_S$, $E_{Su1}$, $E_{Su2}$, $E_{Ac1}$ 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 day, which was used on the day 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 and 5 minutes) is often used in juice pausterization by the food industry (personal communication).

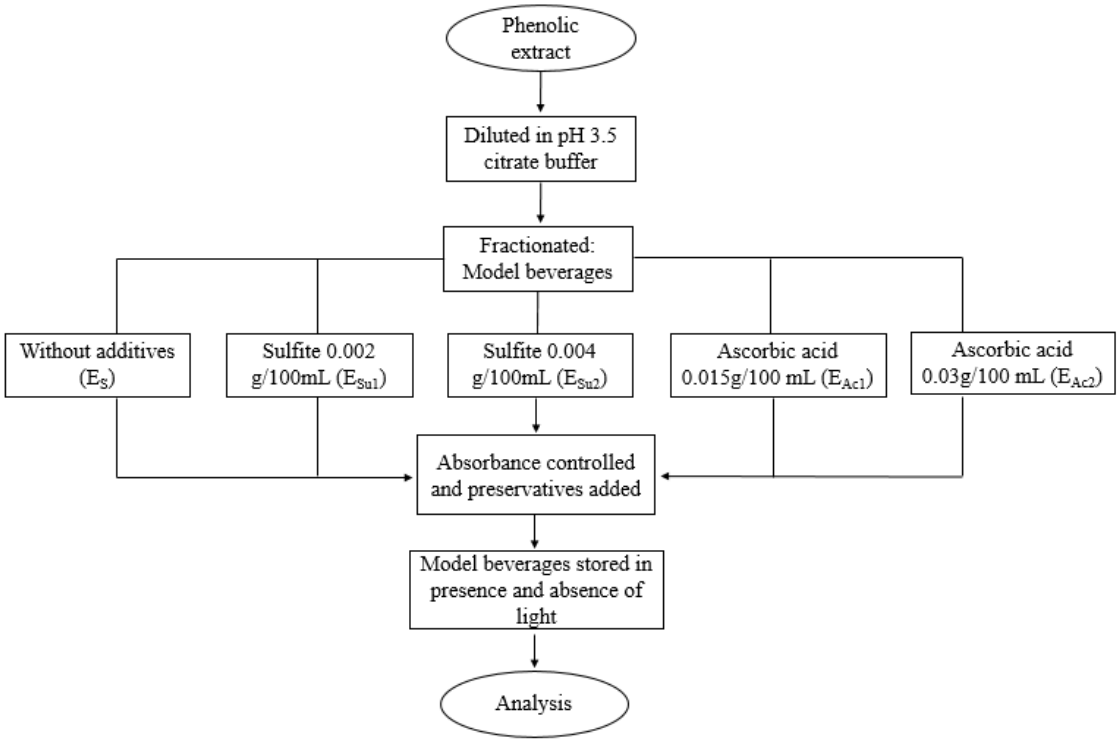



**Figure 12:** Elaboration of model beverages containing ascorbic acid and sulfite

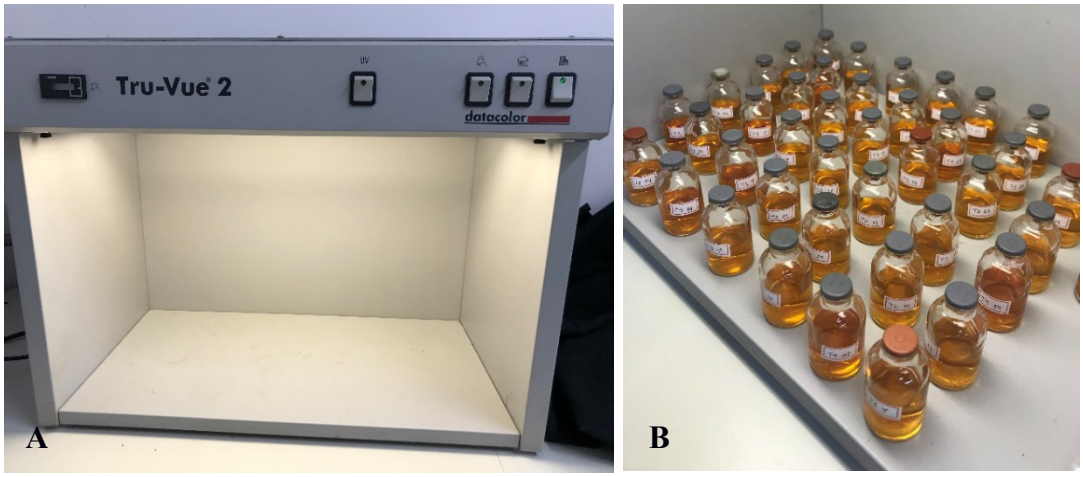



**Figure 13:** Study of the stability of sorghum anthocyanins: (A) Light chamber and (B) Model beverages containing sorghum bran phenolic extract under the incidence of storage light

*4.3.3 Analysis during the stability study*

For the different treatments obtained in both sections (4.3.1 and 4.3.2), analysis of total anthocyanins, total phenolics and color, described in section 4.2, were performed. In addition, for the colorimetric analysis, the global color difference (ΔE*) was calculated during the stability study, according to equation 7. Graphs of the Neperian logarithm (ln) of the initial

absorbance/absorbance ratio *versus* time were constructed, and the degradation constant (kd) was obtained through the slope. From the kd, half-life values were calculated in order to estimate the anthocyanins stability under light exposure, heat and presence of additives (Equation 8).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta \mathrm{b}^*)^2} \quad \text{(Eq. 7)}$$

$$t_{1/2} = \ln 2 / k_d \quad \text{(Eq. 8)}$$

### 4.4 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 5 levels x storage condition in 2 levels), a 3-factor factorial design for the anthocyanins variation and C* and h* colorimetric parameters (additives in 5 levels x condition of storage in 2 levels x time in 4 levels) and a 2-factor factorial design for the ΔE* (additives in 5 levels x storage condition in 2 levels). For the study of stability to heat treatment, a 2-factor factorial design was used for the variation of anthocyanins and for all colorimetric parameters (additives in 5 levels x time in 3 levels). Differences in means were compared using Tukey's post-hoc test. All analyzes adopted a significance level of 5% and were performed using the STATISTICA 13.3 software.

## 5 RESULTS AND DISCUSSION

### 5.1 Characterization of the sorghum bran phenolic extract

Total anthocyanins and phenolic content of the sorghum bran 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 the ones found in some fruits such as jambolan and blackberry (Rigolon et al., 2020; Sari et al., 2012). It confirms that sorghum is a good source of anthocyanins and phenolic compounds. Moreover, it is important to emphasize that the sorghum genotype used is from Brazil and has the highest total anthocyanin content among those evaluated by Emprapa Milho e Sorgo.

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 color with a red-orange tint (Figure 14). The values of h* (hue) and C* (chroma) were 0.89 ± 0.0002 and 53.91 ± 0.10, respectively, indicating the color quality and the intensity. These results differ from phenolic extracts containing anthocyanins obtained from fruits and vegetables since the color depends on the source of anthocyanins (Bridle & Timberlake, 1997; Fernández-López et al., 2013; Sari et al., 2012). 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.

**Figure 14:** Sorghum bran phenolic extract

*5.2. Changes in color, total anthocyanins and phenolics in model beverages containing sulfite and ascorbic acid, under light exposure*

*5.2.1 Changes in total anthocyanin and phenolic contents*

The variation of total anthocyanin content for the model beverages in the presence and absence of light is presented in Figures 15A and 15B, respectively, and in Table 3. There was a significant difference between storage conditions (presence or absence of light) only for model beverages containg sulfite ($E_{Su1}$ and $E_{Su2}$) on days 7 and 14. For the other model beverages ($E_S$, $E_{Ac1}$ and $E_{Ac2}$) there was no significant difference, showing that for both storage conditions, the averages of anthocyanin contents 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 $E_S$, $E_{Ac2}$ $E_{Ac2}$, 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 3).

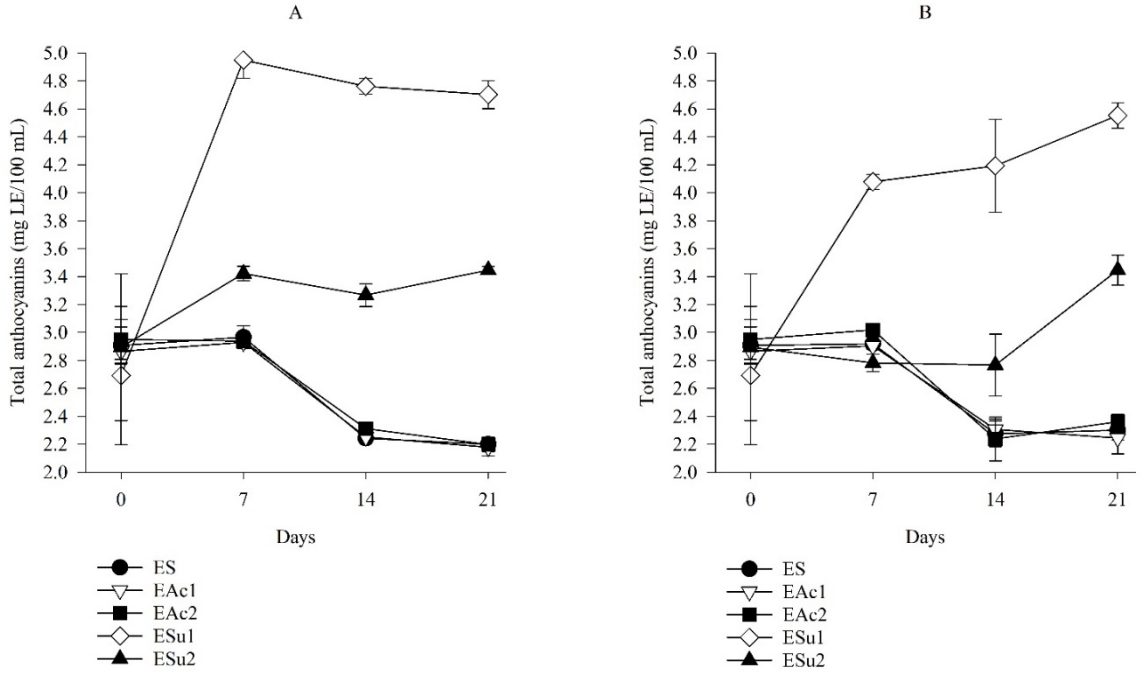



**Figure 15:** Variation of the total anthocyanins contents in model beverages containing sulfite and ascorbic acid: (A): storage for 21 days under light exposure; (B): storage for 21 days in the absence of light.

Results were expressed as means and error bars indicate ± standard deviation (n=3). $E_S$ = 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 sulfite (0.002g/100mL); $E_{Su2}$ = Extract added with sulfite (0.004g/100mL).

**Table 3:** Variation of anthocyanin content in model beverages containing sulfite and ascorbic acid during 21 days of storage under light exposure

| Model beverages | Storage condition | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 7 | | 14 | | 21 | |
| $E_S$ | Light | 2.91 | A ab α | 2.97 | A c α | 2.24 | A c β | 2.20 | A c β |
| | Dark | 2.91 | A **ab** α | 2.92 | A **bc** α | 2.27 | A **c** β | 2.30 | A **c** β |
| $E_{Ac1}$ | Light | 2.87 | A ab α | 2.93 | A c α | 2.25 | A c β | 2.20 | A c β |
| | Dark | 2.87 | A **ab** α | 2.90 | A **bc** α | 2.31 | A **c** β | 2.24 | A **c** β |
| $E_{Ac2}$ | Light | 2.95 | A a α | 2.94 | A c α | 2.31 | A c β | 2.20 | A c β |
| | Dark | 2.95 | A **a** α | 3.02 | A **b** α | 2.24 | A **c** β | 2.36 | A **c** β |
| $E_{Su1}$ | Light | 2.69 | A b γ | 4.95 | A a α | 4.76 | A a αβ | 4.70 | A a β |
| | Dark | 2.69 | A **b** γ | 4.10 | B **a** β | 4.19 | B **a** β | 4.55 | A **a** α |
| $E_{Su2}$ | Light | 2.89 | A ab β | 3.42 | A b α | 3.27 | A b α | 3.45 | A b α |
| | Dark | 2.89 | A **ab** β | 2.78 | B **c** β | 2.77 | B **b** β | 3.45 | A **b** α |

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 (0.015g/100mL); $E_{Ac2}$ = Extract added with ascorbic acid (0.03g/100mL).

Model beverages with addition of sulfite ($E_{Su1}$ and $E_{Su2}$) showed an opposite behavior, in which the total anthocyanin content increased during 21 days of storage in the presence and absence of light (Figures 15A and 15B and Table 3). 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 it increased 69.11% compared to day 0. For the storage under light exposure, the $E_{Su1}$ anthocyanin content was 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, with a significant increase from day 14 until the last day of storage (Table 3). For the same model beverage, in the presence of light, there was a significant increase on the anthocyanin content between day 0 and 7 and it was constant until day 21.

Sulfite is an additive used as a preservative for foods and beverage and its interaction with anthocyanins often leads to bleaching, which is generally reversible, and results from the nucleophilic addition of these molecular species to the C ring of the flavilium cation, leading

to the formation of colorless sulfonates (Cavalcanti et al., 2011; Timberlake & Bridle, 1967). In our study, because of the immediate bleaching effect by addition of sulfite, 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 reversible reaction, the anthocyanin content was higher on the last day of storage compared to the first day. Our results corroborate with the findings of Ojwang & Awika (2010) who observed a higher final absorbance for sorghum 3-deoxyanthocyanidins after 21 days in the presence of sulfite, however, they used an excess of sulfite (molar ratio ~ 1:40, pigment/sulfite).

The recovery of anthocyanins content for sulfite treatments was more evident in light (Figures 15A and 15B) and can be justified by the formation of sulfonate complexes that could possibly copigment with the AH+ increasing color intensity (Ojwang & Awika, 2010). In addition, the 3-deoxyanthocyanins from sorghum have a hydrophobic pyrilium ring and because of that tend to readily self-associate more over time (compared to anthocyanins from fruits and vegetables), protecting the AH+ from further nucleophilic attack after it dissociates from bisulfite ion (Ojwang & Awika, 2010). It was also observed that the recovery of anthocyanins for the ESu1 treatment was more evident than for the $E_{Su2}$ treatment, in both storage conditions (Table 3). This fact may be related to the concentration of sulfite present in the $E_{Su2}$ treatment, twice higher than the concentration found in the $E_{Su1}$ treatment, disfavoring equilibrium of the flavylium cation over the colorless bisulfite adduct (Jurd, 1963).

The behavior of the variation of total phenolics in both storage conditions was similar to that observed for the content of total anthocyanins. For the $E_S$, $E_{Ac1}$ and $E_{Ac2}$ treatments, there was a tendency to decrease the total phenolic content in both storage conditions. The initial phenolic content was 57.66 ± 0.55 mg GAE/mL. For storage in the presence of light, the final levels of total phenolics (mg GAE/mL) for $E_S$, $E_{Ac1}$ and $E_{Ac2}$, respectively, were 55.17 ± 0.48, 54.23 ± 2.43 and 53.07 ± 1.35. As the anthocyanin content decreased for the $E_S$, $E_{Ac1}$ and $E_{Ac2}$ treatments, the same occurred for the total phenolic content. It should be noted that, in addition to anthocyanins, sorghum bran also has other phenolic compounds, such as phenolic acids and flavonoids (Awika et al., 2005). For the $E_{Su1}$ and $E_{Su2}$, similar to anthocyanins behavior, the phenolic content increased in both storage conditions for 21 days. Just as the anthocyanin content was higher for the $E_{Su1}$ treatment (lower sulfite concentration), the same was true for the total phenolic content, with final values of 111.67 ± 3.72 and 114.72 ± 2.47 mg GAE/100mL for storage in the presence and absence of light, respectively. The increase in the total phenolic content was less pronounced for the $E_{Su2}$ treatment, which, again, may be related to the higher concentration of added sulfite.

*5.2.2 Kinetics of anthocyanin degradation*

The degradation kinetics of anthocyanins is presented in Table 4. Values of the degradation constant (k), half-life ($t_{1/2}$) and percentage of degradation of sorghum anthocyanins in the presence of ascorbic acid (both concentrations) did not differ in the presence or absence of light. For the treatments with the addition of sulfite ($E_{Su1}$ and $E_{Su2}$), no kinetics of degradation were presented since there was a recovery of the total anthocyanin content over time.

It was observed that the percentage of degradation 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 4). There was no significant difference in the degradation of 3-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 colorless forms (i.e. the carbinol pseudobase and chalcone) (Dyrby et al., 2001).

**Table 4:** Anthocyanins degradation kinetics in model beverages containing ascorbic acid for 21 days under light exposure

| | | Treatments | | | |
|---|---|---|---|---|---|
| | **Condition** | **$E_S$** | **$E_{Ac1}$** | **$E_{Ac2}$** | **Means** |
| **k ($x10^{-3}$)** | Light | 0.55 | 0.54 | 0.58 | $0.56 \pm 0.02^{A}$ |
| | Dark | 0.46 | 0.49 | 0.44 | $0.46 \pm 0.02^{A}$ |
| | **Means** | $0.51 \pm 0.06^{a}$ | $0.52 \pm 0.04^{a}$ | $0.51 \pm 0.10^{a}$ | |
| **$t_{1/2}$ (dias)** | Light | 54.70 | 53.22 | 50.38 | $52.76 \pm 2.20^{A}$ |
| | Dark | 66.29 | 59.68 | 66.94 | $64.30 \pm 4.03^{A}$ |
| | **Means** | $60.50 \pm 8.19^{a}$ | $56.44 \pm 4.57^{a}$ | $58.66 \pm 11.73^{a}$ | |
| **%degradation** | Light | 24.16 | 23.99 | 25.38 | $24.51 \pm 0.76^{A}$ |
| | Dark | 20.72 | 21.74 | 19.87 | $20.77 \pm 0.94^{A}$ |
| | **Means** | $22.44 \pm 2.43^{a}$ | $22.86 \pm 1.60^{a}$ | $22.63 \pm 3.90^{a}$ | |

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 between the factos storage condition and additive. $E_S$ = Extract without additives; $E_{Ac1}$ = Extract added with ascorbic acid (0.015g/100mL); $E_{Ac2}$ = Extract added with ascorbic acid (0.03g/100mL).

The lack of substitution at C-3 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 to nucleophilic attack and hydration, which is a primary mechanism for the structural transformation of anthocyanins in solution to colorless 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 color 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 and that methoxylation at position 5, and not position 7, was key to the improved stability. In our study, since it was used a crude phenolic extract, the great stability to light could also be related to the copigmentation and complexation of anthocyanins with other extracted phenolic constituents, as reported by Awika et al (2004b).

The stability of sorghum 3-deoxyanthocyanins to light is advantageous, considering that common anthocyanins (i.e. cyanidin and delfinidine 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 color 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 24h 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 colorant can be applied to foods and non-food products commonly exposed to fluorescent light.

Regarding the effect of the ascorbic acid, the degradation percentages, respectively, for the treatments added of 0.015g/100 mL ($E_{Ac1}$) and 0.03g/100 mL of ascorbic acid ($E_{Ac2}$) in the presence of light were 23.99 and 25.38%, not differing from each other, indicating that the variation in the ascorbic acid concentration did not interfere in the degradation of sorghum anthocyanins (Table 4). The same behavior was observed for treatments EAc1 and EAc2 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 $E_{Ac2}$ in both storage conditions did not differ from the treatment $E_S$ (control), indicating the stability of 3-deoxyanthocyanins to light exposure and to 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-deoxythocyanins showed stability to ascorbic acid which can also be related to their different structure: the lack of –OH group in C-3 position. Furthermore, crude sorghum extract contains other more stable forms of 3-deoxyanthocyanins (glycosides, acylglycosides, etc.) and it is also likely to contain other phenolic compounds that could act as co-pigments, enhancing the anthocyanin stability in the presence of ascorbate degradation products (Ojwang & Awika, 2008). Our results corroborate with earlier findings of Ojwang &

Awika (2008) who reported that, in the presence of 500 mg/L of ascorbic acid, red cabbage pigment lost 85% of its color while sorghum bran extract lost only 31% of its color after 21 days at pH 2.0 under fluorescent laboratory lighting. The concentration of ascorbic acid used by these authors was superior to the ones 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.

*5.2.3 Changes in color of model beverages containing sorghum anthocyanins*

Variations of the chroma (C *) and hue (h*) coordinates in model beverage systems are presented in Table 5. By color coordinates observed and calculated from the values of a* and b* at the beginning of this experiment, 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 colors, respectively, with plenty of light (high value of L*); high values C*, indicating that the treatments had pure and intense colors; and low values of h*, indicating shade that tends to red, relating to the anthocyanin pigment present in sorghum bran.

**Table 5:** Variation of Chroma (C*) and hue (h*) coordinates in model beverages containing sulfite and ascorbic acid for 21 days under light exposure

| Model beverages | | Storage condition | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | | 7 | | 14 | | 21 | |
| C* | $E_S$ | Light | 49.51 | A c α | 49.14 | A b α | 46.66 | B c β | 44.80 | B c γ |
| | | Dark | 49.51 | A **c** α | 49.10 | A **c** αβ | 48.40 | A **c** β | 47.24 | A **c** γ |
| | $E_{Ac1}$ | Light | 51.78 | A b α | 48.82 | B b β | 46.60 | B c γ | 44.50 | B c δ |
| | | Dark | 51.78 | A **b** α | 49.82 | A **c** β | 48.23 | A **cd** γ | 47.10 | A **c** δ |
| | $E_{Ac2}$ | Light | 51.80 | A b α | 49.04 | A b β | 46.62 | B c γ | 44.10 | B c δ |
| | | Dark | 51.80 | A **b** α | 49.63 | A **c** β | 47.42 | A **d** γ | 47.20 | A **c** γ |
| | $E_{Su1}$ | Light | 55.01 | A a α | 55.20 | B a α | 53.48 | B a β | 50.80 | B a γ |
| | | Dark | 55.01 | A **a** β | 56.04 | A **a** α | 55.18 | A **a** αβ | 54.39 | A **a** β |
| | $E_{Su2}$ | Light | 51.63 | A b α | 48.36 | B b γ | 50.65 | A b β | 48.62 | B b γ |
| | | Dark | 51.63 | A **b** α | 51.72 | A **b** α | 51.32 | A **b** α | 50.18 | A **b** β |
| h* | $E_S$ | Light | 1.17 | A b α | 1.15 | B a β | 1.12 | B a γ | 1.09 | B a δ |
| | | Dark | 1.17 | A **b** α | 1.17 | A **a** α | 1.15 | A **a** β | 1.13 | A **a** γ |
| | $E_{Ac1}$ | Light | 1.18 | A b α | 1.15 | B a β | 1.12 | B a γ | 1.09 | B a δ |
| | | Dark | 1.18 | A **b** α | 1.17 | A **a** β | 1.15 | A **a** γ | 1.13 | A **a** δ |
| | $E_{Ac2}$ | Light | 1.18 | A b α | 1.15 | B a β | 1.12 | B a γ | 1.09 | B a δ |
| | | Dark | 1.18 | A **b** α | 1.17 | A **a** α | 1.16 | A **a** β | 1.13 | A **a** γ |
| | $E_{Su1}$ | Light | 1.14 | A c α | 1.04 | B c β | 0.97 | B c γ | 0.93 | B c δ |
| | | Dark | 1.14 | A c α | 1.08 | A **b** β | 1.02 | A **c** γ | 1.00 | A **c** δ |
| | $E_{Su2}$ | Light | 1.21 | A a α | 1.13 | B b β | 1.07 | B b γ | 1.03 | B b δ |
| | | Dark | 1.21 | A **a** α | 1.17 | A **a** β | 1.13 | A **b** γ | 1.09 | A **b** δ |

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 (0.015g/100mL); $E_{Ac2}$ = Extract added with ascorbic acid (0.03g/100mL).

Chroma values (C*) decreased over 21 days, for all treatments in both storage conditions, with ESu1 being the only exception in which the value in day 0 did not differ from day 21 (Table 5). This fact could be related to the recovery of anthocyanins which was more evident for the ESu1 model beverage. When analyzing 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 color (decrease in C* values) as a consequence of the loss of anthocyanins and formation of degradation compounds such as colorless pseudobase or carbinol (Cevallos-Casals & Cisneros-Zevallos, 2004). Regarding the presence of food additives, on days 7 and 21, there was no significant difference for the values of C* between the model beverages $E_S$, $E_{Ac1}$ and $E_{Ac2}$, and the beverages containing sulfite presented different values between them and among the other treatments. The decrease in saturation was less pronounced for treatments containing sulfite ($E_{Su1}$ and $E_{Su2}$) since, as demonstrated for the stability of anthocyanins, there was a reaction to reverse the whitening effect caused by the addition of sulfite, that is, less change in the intensity of color in these treatments.

The hue values (h*) decreased from day 7, between the storage conditions for all treatments (Table 5). This fact indicates the change in shade of the original color. There was no significant difference in the h* parameter between 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 on 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 was reported on day 21. For all treatments in the light there was a decrease in h* from day 7 with lowest values also on day 21. The decrease in the values of h* and C* 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 color variation (ΔE*) is presented in Table 6. 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 color of the beverages between the beginning and the end of the experiment, since an ΔE* of 1.0 represents the threshold of perception of color difference to the human eye (Gonnet, 1998). However, small color differences, even if detected by vision, are not sufficient to mischaracterize a product or negatively influence its acceptance. Changes in h*, C* and ΔE* are in accordance with results reported on model beverage colored with Andes Berry anthocyanin powder after storage for 71 days at 16°C in daylight (Estupiñan et al., 2011).

The $E_S$ treatment (without additives) showed the lowest overall color variation in both conditions, with values of 6.29 and 3.59 in the storage in the presence and absence of light, respectively (Table 6). The highest values of global color difference were for the treatments $E_{Su1}$ and $E_{Su2}$ in the presence of light, with values of 14.84 and 11.74, respectively; 9.74 and

7.97 in the absence of light, respectively. For sulfite 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). This overall difference can be seen in Figure 16 in which the color of the treatments added with sulfite on the last day of storage is noticeably darker when compared to the other treatments, in the presence of light.

**Table 6:** Global color variation in model beverages containing sulfite and ascorbic acid for 21 days under light exposure

| Treatments/Model beverages | ΔE* | |
|---|---|---|
| | **Light** | **Dark** |
| $E_S$ | $6.29 \pm 0.335^{Da}$ | $3.59 \pm 0.384^{Db}$ |
| $E_{Ac1}$ | $8.98 \pm 0.442^{Ca}$ | $5.42 \pm 0.119^{Cb}$ |
| $E_{Ac2}$ | $9.09 \pm 0.414^{Ca}$ | $5.29 \pm 0.195^{Cb}$ |
| $E_{Su1}$ | $14.84 \pm 0.282^{Aa}$ | $9.74 \pm 0.128^{Ab}$ |
| $E_{Su2}$ | $11.74 \pm 0.267^{Ba}$ | $7.97 \pm 0.115^{Bb}$ |

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. $E_S$ = 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 sulfite (0.002g/100mL); $E_{Su2}$ = Extract added with sulfite (0.004g/100mL).

### *5.3. Changes in color, total anthocyanins and phenolics in model beverages subjected to heat treatment*

#### *5.3.1 Changes in total anthocyanin and phenolic contents*

The total anthocyanin and phenolic contents of model beverages before and after heat treatment at 80 °C (0, 5 and 25 min) are presented in Table 7. It was observed that the total anthocyanin content did not differ ($P > 0.05$) for the same treatment over time and among different treatments over time.

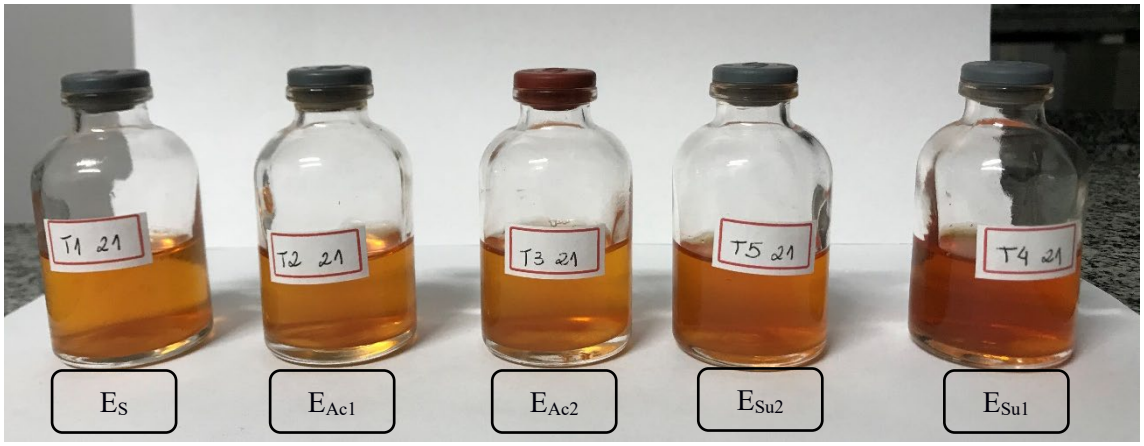



**Figure 16:** Model beverages elaborated with sorghum bran phenolic extract, containing additives (sulfite and ascorbic acid) at day 21 of storage under light exposure

$E_S$ = 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 sulfite (0.002g/100mL); $E_{Su2}$ = Extract added with sulfite (0.004g/100mL).

**Table 7:** Total anthocyanins and phenolic contents of model beverages containing ascorbic acid and sulfite after heat treatment at 80 °C for 0, 5 and 25 min

| **Treatments** | **Total anthocyanins (mg LE/100 mL)** | | | |
|---|---|---|---|---|
| | 0 min | 5 min | 25 min | **Means** |
| $E_S$ | 2.689 | 2.604 | 2.658 | $2.650 \pm 0.04^{A}$ |
| $E_{Ac1}$ | 2.604 | 2.627 | 2.629 | $2.620 \pm 0.01^{A}$ |
| $E_{Ac2}$ | 2.621 | 2.658 | 2.735 | $2.671 \pm 0.06^{A}$ |
| $E_{Su1}$ | 2.655 | 2.672 | 2.652 | $2.660 \pm 0.01^{A}$ |
| $E_{Su2}$ | 2.575 | 2.612 | 2.615 | $2.601 \pm 0.02^{A}$ |
| **Means** | $2.629 \pm 0.04^{a}$ | $2.635 \pm 0.03^{a}$ | $2.658 \pm 0.05^{a}$ | |
| **Treatments** | **Total phenolics (mg GAE/100 mL)** | | | |
| | 0 min | 5 min | 25 min | **Means** |
| $E_S$ | 62.88 | 62.86 | 65.25 | $63.66 \pm 1.37^{A}$ |
| $E_{Ac1}$ | 62.86 | 63.41 | 63.69 | $63.32 \pm 0.42^{A}$ |
| $E_{Ac2}$ | 62.15 | 63.99 | 64.60 | $63.58 \pm 1.28^{A}$ |
| $E_{Su1}$ | 63.51 | 63.08 | 65.95 | $63.18 \pm 1.55^{A}$ |
| $E_{Su2}$ | 62.78 | 62.54 | 65.04 | $63.45 \pm 1.38^{A}$ |
| **Means** | $62.84 \pm 0.48^{b}$ | $63.17 \pm 0.55^{b}$ | $64.91 \pm 0.83^{a}$ | |

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 between the factors additive and time. $E_S$ = 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 sulfite (0.002g/100mL); $E_{Su2}$ = Extract added with sulfite (0.004g/100mL).

Sorghum 3-deoxyanthocyanins once again showed good stability with respect to additives and the combination with heat treatment commonly used in the juice industry. The 3-desoxyanthocyanins resist fragmentation during thermal treatment, they have greater deprotonation rate constant than hydration rate constant, hence convert less to the colorless 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 highest thermal stability at pH 1 and 2, with >80% color retention and 68–78% color retention at pH 3-6. In the same study, the crude extract retained 89% of color after 95°C/2 h heat treatment.

For the phenolic content, it was observed similar behavior of total anthocyanins, without significant changes (Table 7). However, there was a slight, but significant increase in total phenolic content after heat treatment at 80 °C for 25 min. The hypothesis that possibly explains this behavior is that the high temperature used can lead to the formation of thermal degradation compounds, such as Maillard reaction products that have a reducing capacity, increasing the total phenolic content (Eichner, 1981).

For sulfite treatments ($E_{Su1}$ and $E_{Su2}$), unlike their behavior 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 7). The bleaching reaction by the sulfite is reversible over time and, since it was used only 5 and 25 min in the heat stability study, there was no recovery of the anthocyanin content.

*5.3.2 Changes in color of model beverages containing sorghum anthocyanins*

Color stability of sorghum anthocyanins after heat treatment was demonstrated by colorimetric analysis (Tables 8 and 9). The stability of sorghum anthocyanins after heat treatment can also be related to color stability. Regarding chroma (C*), there was no significant difference for each treatment before and after heat treatment for 25 min (Table 8). Moreover, the hue (h*) did not differ during the heat treatment for 5 and 25 min for the same treatment and between different treatments (Table 9), indicating that there was no change in shade of the model beverage systems. Such results demonstrate once again the color stability of sorghum anthocyanins to heat treatment (Akogou et al., 2018; Yang et al., 2014).

Global color variation after heat treatment is presented in Table 9. 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 color 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).

**Table 8:** Variation of Chroma (C*) coordinate in model beverages containing sulfite and ascorbic acid after heat treatment at 80 ºC (0, 5 and 25 min)

| **Treatments** | **C*** | | |
|---|---|---|---|
| | 0 min | 5 min | 25 min |
| $E_S$ | $58.95 \pm 0.11^{Aab}$ | $58.41 \pm 0.85^{Bb}$ | $59.11 \pm 0.27^{Aa}$ |
| $E_{Ac1}$ | $59.10 \pm 0.04^{Ab}$ | $59.78 \pm 0.06^{Aa}$ | $59.35 \pm 0.37^{Aab}$ |
| $E_{Ac2}$ | $59.01 \pm 0.003^{Aa}$ | $59.56 \pm 0.24^{Aa}$ | $59.36 \pm 0.33^{Aa}$ |
| $E_{Su1}$ | $59.04 \pm 0.03^{Ab}$ | $59.81 \pm 0.24^{Aa}$ | $59.39 \pm 0.41^{Aab}$ |
| $E_{Su2}$ | $59.47 \pm 0.04^{Aa}$ | $59.86 \pm 0.14^{Aa}$ | $59.32 \pm 0.28^{Aa}$ |

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. $E_S$ = 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 sulfite (0.002g/100mL); $E_{Su2}$ = Extract added with sulfite (0.004g/100mL).

**Table 9:** Global color variation (ΔE*) and variation of hue (h*) in model beverages containing sulfite and ascorbic acid after heat treatment at 80 ºC (0, 5 and 25 min)

| **Treatments** | **ΔE*** | | |
|---|---|---|---|
| | 5 min | 25 min | **Means** |
| $E_S$ | 1.01 | 2.19 | $1.60 \pm 0.83^{A}$ |
| $E_{Ac1}$ | 0.91 | 2.06 | $1.49 \pm 0.81^{A}$ |
| $E_{Ac2}$ | 0.87 | 1.99 | $1.43 \pm 0.79^{A}$ |
| $E_{Su1}$ | 0.96 | 1.79 | $1.38 \pm 0.59^{A}$ |
| $E_{Su2}$ | 0.72 | 1.88 | $1.30 \pm 0.82^{A}$ |
| **Means** | $0.89 \pm 0.11^{b}$ | $1.98 \pm 0.15^{a}$ | |

| **Treatments** | **h*** | | | |
|---|---|---|---|---|
| | 0 min | 5 min | 25 min | **Means** |
| $E_S$ | 1.26 | 1.25 | 1.22 | $1.24 \pm 0.02^{A}$ |
| $E_{Ac1}$ | 1.25 | 1.24 | 1.22 | $1.24 \pm 0.02^{A}$ |
| $E_{Ac2}$ | 1.25 | 1.24 | 1.23 | $1.24 \pm 0.01^{A}$ |
| $E_{Su1}$ | 1.25 | 1.24 | 1.22 | $1.24 \pm 0.02^{A}$ |
| $E_{Su2}$ | 1.25 | 1.24 | 1.23 | $1.24 \pm 0.01^{A}$ |
| **Means** | $1.25 \pm 0.002^{a}$ | $1.24 \pm 0.002^{a}$ | $1.22 \pm 0.001^{a}$ | |

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 between the factors additive and time. $E_S$ = 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 sulfite (0.002g/100mL); $E_{Su2}$ = Extract added with sulfite (0.004g/100mL).

## 6 CONCLUSION

Crude sorghum 3-deoxyanthocyanins added in model beverage systems (pH 3.5) have great stability during storage for 21 days under fluorescent light exposure, presence of ascorbic acid and sulfite in concentrations commonly found in Brazilian beverages, and heat treatment (80ºC for 5 or 25 min). Therefore, sorghum bran phenolic extract, containing 3-deoxyanthocyanins, has potential to be used as natural food colorant and nutraceutical ingredient in many beverage products.

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