Optimization of extraction, characterization, and stability of the natural pigment from sorghum genotype SC 319

Otimização da extração, caracterização e estabilidade do corante natural de sorgo de genótipo SC 319

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ABSTRACT

Darker pericarp sorghum genotypes have high levels of anthocyanins: natural pigments that have antioxidant potential and may serve as a natural alternative dye to replace synthetic colorants. Thus, the objective of this work was to obtain a powder dye from sorghum pericarp genotype SC 319, quantifier total phenolic compounds, total anthocyanins, and color. In addition, evaluate the stability during storage at room temperature for 30 days and test an application in gelatin gummies. There was no significant difference for total phenolic compounds during all days of storage, with values between 36.80 and 37.97 mg GAE.g^{-1}. For total anthocyanins, despite the variation in

levels during storage, the values obtained on the first day (2.407 mg Eq. Lut.g^{-1}) and on the last day (2.379 mg Eq. Lut.g^{-1}) did not differ. There was a significant difference in all colorimetric axes and the reddish hue increased during storage, ranging from 7.04 to 9.11. For the gelatin gummies, compared to the powder dye, the anthocyanins and phenolic contents were lower, but the red hue presented a high value (28.9). Therefore, it was possible to obtain a stable powder natural dye from sorghum pericarp that can provide color and bioactive compounds to foods.

Keywords: anthocyanins, phenols contents, natural food coloring, dye.

RESUMO

Genótipos de sorgo de pericarpo mais escuro possuem altos teores de antocianinas: pigmentos naturais que apresentam potencial antioxidante e podem servir como uma alternativa de corante natural para substituir os corantes sintéticos. Assim, o objetivo deste trabalho foi obter um corante do pericarpo de sorgo de genótipo SC 319 em pó, caracterizá-lo com relação à cor, compostos fenólicos e antocianinas totais. Além de avaliar sua estabilidade durante o armazenamento em temperatura ambiente por 30 dias e testar a aplicação em balas de gelatina. Não houve diferença significativa para os compostos fenólicos durantes todos os dias de armazenamento, com valores entre 36,80 e 37,97 mg GAE.g^{-1}. Para as antocianinas, apesar da variação dos teores ao longo da estocagem, os valores obtidos no primeiro dia (2,407 mg Eq. Lut.g^{-1}) e no último (2,379 mg Eq. Lut.g^{-1}) não diferiram entre si. Houve diferença significativa em todos os eixos colorimétricos e a tonalidade avermelhada aumentou durante o armazenamento, variando de 7,04 a 9,11. Para a bala de gelatina, em comparação ao corante em pó, os teores de antocianinas e fenólicos foram mais baixos, mas a tonalidade vermelha apresentou valor elevado (28,9). Portanto, foi possível obter um corante natural em pó estável extraído do pericarpo de sorgo que pode conferir cor e compostos bioativos aos alimentos.

Palavras-chave: antocianinas, compostos fenólicos, pigmentos naturais.

1 INTRODUTION

Color and appearance are determining factors for consumer acceptance of food products. Therefore, synthetic and natural dyes are commonly used by the food industry in order to enhance color or restore it when lost during processing (Constant et al., 2002; Retondo; Faria, 2009).

The dyes allowed in different countries vary considerably due to the diversity of substances with coloring power. Brazil has the largest number of authorized synthetic dyes (Castro et al., 2021). Because of the increase in the number of these compounds and their wide use in foods and beverages, it was necessary to control their applications and carry out studies on implications for human health (Prado; Godoy, 2003). According to Shahid and Mohammad (2007), synthetic dyes have lower production costs, greater stability, and dyeing capacity, however, due to their toxicity, the number of these

permitted additives is decreasing, increasing the demand for natural dyes (Zanoni; Yamanaka, 2016; Pazmiño-Durán et al., 2001). Thus, many works have been developed to evaluate the toxicity of these additives (El-Wahab; Moram, 2013; Amim et al., 2010; Mpountoukas et al., 2010; Lau et al., 2005). Implications of this toxicity can lead to allergies and cancer risks (Amchova et al., 2015).

In this regard, anthocyanins are presented as an alternative natural dye, since they constitute the largest group of water-soluble pigments found in the plant kingdom (Bridle; Timberlake, 1997), ranging from red to blue coloring in many fruits and vegetables (Mazza; Miniati, 1993; Volp et al., 2008). This natural pigment has a high antioxidant potential, preventing auto-oxidation and lipid peroxidation in biological systems (Kuskoski, 2004). However, anthocyanins can undergo color change due to chemical reactions of food products, since they have chromophore groups sensitive to changes in pH (Constant et al., 2002).

Despite the sorghum (*Sorghum bicolor* L.) is widely used for animal feed, it has phytochemical compounds with effects in the prevention of chronic non-communicable diseases, such as cardiovascular diseases, diabetes, obesity, and cancer (Dykes et al., 2009). Among these compounds, anthocyanin pigments stand out, mainly in darker pericarp sorghum grains, such as black, brown, and red (Queiroz et al., 2014). Additionally, the main anthocyanins present in this grain are denominated 3-deoxyanthocyanidins, which are rarely found in nature and do not have a hydroxyl group in the C-3 position (Clifford, 2000). This characteristic makes sorghum anthocyanins more stable to pH variations, reflecting the coloring potential of the grain, which is still little explored (Awika et al., 2004; Dykes et al., 2009).

Thus, the objective of this work was to obtain a natural dye from sorghum pericarp by extraction in acidified water, evaluate the phenolic compounds, anthocyanins, color, and the stability of the lyophilized extract (dye powder) during storage at room temperature for 30 days. In addition, an application test in gelatin candy was elaborated.

2 MATERIAL AND METHODS

Brown pericarp sorghum grains of SC 319 genotype cultivated at Embrapa Maize & Sorghum (Sete Lagoas, MG, Brazil) were used. The pericarp removal from the grain was carried out in a rice processing machine (Nogueira Cimag, rural model, series B-7, 760 rpm), ground in a ball mill grinder (Retsch MM200 model) and granulometry was standardized in a sieve of 125 µm.

2.1 OPTIMIZATION OF ANTHOCYANIN EXTRACTION

For the extraction of anthocyanin compounds from the sorghum pericarp, different concentrations of mass:volume were evaluated: 1:100; 2:100; 3:100; and 4:100 (pericarp (g) in acidified water (mL) 3% citric acid).

The extractions were carried out in erlenmeyer flasks in a water bath (Solab, model Dubnoff SL-157) at 90°C, under agitation (200 rpm/2 h), according to Queiroz et al. (2014). Subsequently, the extracts were centrifuged (1710.54 rfc/15 min) (Ecco model Superior IV-B) and analyzed for color and total anthocyanin content.

2.2 DYE POWDER AND STABILITY STUDY

Extraction was performed following the proportion that resulted in the highest anthocyanin content (1:100). Therefore, 15 g of sorghum pericarp were extracted in 1500 mL of acidified water. The extract was vacuum filtered through quantitative filter paper (125 mm) using Prismatec pump (model 131) and stored in amber glass flasks with lids under refrigeration until drying and/or characterization analysis. The volume of extract obtained was equally arranged in 300 mL trays and frozen at -18°C for 48 h. The stainless-steel trays were attached to the lyophilizer (Terroni model LS6000) under operating conditions of 120 mHg at -45ºC. After the complete water loss, the solid formed was macerated with a mortar and pestle to obtain a powder.

The powder dye was stored in a transparent glass bottle with a hermetic lid and stored at room temperature (25°C ± 1°C), monitored by a thermos-hygrometer (Ion Therm model 500). Color, phenolic compounds, and anthocyanins were analyzed at 0, 7, 14, 21 and 30 days of storage.

2.3 PHYSICOCHEMICAL ANALYSIS

2.3.1 Total Anthocyanins

The analysis of total anthocyanins was performed according to Yang (2010) by reading the absorbance in quartz cuvettes using a spectrophotometer (Instrutherm model UV 2000A) at 480 nm. Total anthocyanins contents were expressed in mg Eq. Lut.g^{-1}, which were calculated based on the equation $C(mg\ Eq.of\ luteolinidin\ g^{-1}) = (A/\varepsilon * 10^3) * MM * V * Fd$, where A is the absorbance of the sample, ε represents the molar extinction coefficient of luteolinidin (29,157), MM is the molar mass of luteolinidin

(270 g.mol-1), V corresponds to the extraction volume in liters and Fd is the dilution factor.

For the study of extraction optimization, anthocyanins analysis was performed under the extract itself. The monitoring of the stability of anthocyanin compounds of the powder dye was carried out by dissolving 0.25 g of powder in 25 mL of water (1:100). This same mass:volume ratio was used to read anthocyanins for the gummy candy, however the solution was heated to 60°C to liquefy the gelatin.

2.3.2 Total Phenolic Compounds

Total phenolic compounds were analyzed using the Folin-Ciocalteu method of Kaluza et al. (1980) with modifications. About 0.25 g of the dye powder was dissolved in 25 ml of water, taking an aliquot of 0.1 ml. Into this, 1.1 mL of water, 0.4 mL of Folin-Ciocalteu reagent, and 0.9 mL of 0.5 M ethanolamine were added. The system was rested at room temperature for 20 min. The absorbance reading was carried out in a quartz cuvette in a spectrophotometer (Instrutherm model UV 2000A) at a wavelength at 600 nm. For the extract obtained with acidified water, the analysis was carried out by directly removing the 0.1 mL aliquot adding the other reagents. For the analysis of the gummy candy, the aliquot used was taken from dissolving 0.25 g of candy in 25 mL of water at 60°C.

2.3.3 Color

The colorimetric evaluation was determined using the Cielab system (L*, a* and b*), in a Chroma Meter colorimeter (model cr400), using cuvettes containing 15 mL of the extract or the dye solution used for anthocyanin analysis. For the analysis of the gummies, the reading was performed directly under the product. A white background was standardized for all samples. With the values of the L*, a* and b* parameters, the total color difference (ΔE*) of the diluted powder dye in relation to the color of the extract to be lyophilized was calculated, through the square root of the sum of the squared parameters.

2.3.4 Moisture

The powder dye moisture was determined by the gravimetric method, based on the removal of water by heating in an oven at 105°C for 6 hours, until constant weight (Instituto Adolfo Lutz, 2005).

2.4 DEVELOPMENT OF GELATIN GUMMY WITH SORGHUM DYE POWDER

The gelatin gummies were prepared at the New Products Development Laboratory in the Federal University of São João del-Rei, Campus Sete Lagoas. For its preparation, the formulation presented in Table 1 was followed.

Table 1. Formulation of naturally colored gelatin candies with sorghum dye powder.

Ingredients	**Quantity**
Gelatin	12 g
Sugar	60 g
Glucose syrup	45 g
Sorghum dye powder	5 g
Water	60 mL
Strawberry flavor	1 mL

The powder dye was dissolved in water (85°C) and fractionated into two equal volumes. In one of the fractions, gelatin was added, remaining in a water bath at 60°C for 20 minutes to hydrate. In the other, sugar was dissolved, glucose syrup was added and the syrup was boiled. The two fractions were mixed until all the gelatin was dissolved and the strawberry essence was added. Then, the syrup formed was transferred to the silicone bullet molds. The candies were kept in a refrigerator (6ºC/2h), acquiring a characteristic consistency. Color, total anthocyanins and phenolic compounds were analyzed.

2.5 STATISTICAL ANALYSIS

All analyzes were performed in triplicate. The data were analyzed by ANOVA and the comparison of means was performed by Tukey's test at 5% probability, with the aid of the statistical program SISVAR.

3 RESULTS AND DISCUSSION

3.1 OPTIMIZATION OF ANTHOCYANIN COMPOUNDS EXTRACTION

Table 2 shows the results of total anthocyanins contents for the 4 treatments evaluated in the extraction optimization process, which ranged from 1.15 to 1.73 mg Eq. Lut.g^{-1}.

Table 2. Total anthocyanins of the extracts obtained from sorghum pericarp.

Treatments (g of pericarp: mL de solution)	**Total Anthocyanins*** (mg Eq. de Lut.g^{-1})
T1 (1:100)	1.73 ± 0.15 ^{a}
T2 (2:100)	1.43 ± 0.18 ^{ab}
T3 (3:100)	1.28 ± 0.10 ^{b}
T4 (4:100)	1.15 ± 0.22 ^{b}

*Means ± standard deviation followed by the same letter do not differ from each other by Tukey's test at 5% probability.

Oliveira et al. (2014) found values between 0.04 and 0.8 mg Eq. Lut.g^{-1} for total anthocyanins levels in 90 accessions of sorghum grains from the Active Germplasm Bank maintained at Embrapa Maize & Sorghum, which were extracted in acidified methanol solution (1% HCl). These values were lower than those obtained in this work, indicating that SC 319 genotype is a good source of anthocyanins.

Treatment T1 was significantly higher than T3 and T4 in total anthocyanins content. The 1:100 ratio was also used by Queiroz et al. (2014) to evaluate different solvents in the extraction of anthocyanin compounds from sorghum glumes. The values obtained for extraction in acidified water (3% citric acid) and acidified methanol (1% HCl) did not differ, which proves the efficiency of extraction in water, allowing the application of anthocyanin compounds in the food industry.

Regarding the colorimetric evaluation, the results obtained are shown in Table 3. Treatment T1 showed the highest values for luminosity (L*) and axis b* (37.62 and 25.74, respectively), indicating greater brightness and yellow hue. For the parameter a*, there was no significant difference (values from 13.08 to 15.79), indicating a reddish hue in all extracts.

Table 3. Colorimetric evaluation of extracts obtained from sorghum pericarp.

Treatments (g of pericarp/mL de solution)	**Colorimetric parameters**		
	L*	**a***	**b***
T1 (1:100)	37.62 ± 1.11 ^{a}	15.79 ± 0.82 ^{a}	25.74 ± 2.10 ^{a}
T2 (2:100)	33.08 ± 1.01 ^{b}	15.45 ± 1.53 ^{a}	17.63 ± 2.57 ^{b}
T3 (3:100)	31.66 ± 1.35 ^{c}	13.93 ± 2.97 ^{a}	14.30 ± 3.45 ^{c}
T4 (4:100)	31.88 ± 0.48 ^{bc}	13.08 ± 2.58 ^{a}	11.88 ± 2.36 ^{d}

*Means ± standard deviation followed by the same letter do not differ from each other by Tukey's test at 5% probability.

Therefore, the best treatment for extracting anthocyanin compounds from the sorghum pericarp was T1, using the ratio 1:100 (mass:volume).

3.2 CHARACTERIZATION OF THE EXTRACT AND DYE POWDER

The image of the powder dye produced by lyophilization of the anthocyanin extract of the sorghum pericarp SC 319 was shown in Figure 1, which presented an average moisture content of 9.2%.

Figure 1. Image of dye powder extracted from sorghum pericarp.

The results of total anthocyanins and total phenolic compounds in the extract and dye powder are presented in Table 4.

Table 4. Total anthocyanins and total phenolic compounds in sorghum pericarp extract, dye powder and diluted dye (1:100).

	Extract (g of pericarp)	**Dye powder** (g of powder)	**Diluted dye*** (mL)
Total anthocyanins (mg Eq. lut)	1.31 ± 0.13	2.42 ± 0.016	0.605 ± 0.003
Total phenolic compounds (mg GAE)	31.54 ± 1.65	36.96 ± 2.05	9.24 ± 0.53

Awika et al. (2004) evaluated the content of total anthocyanins in grains and bran of black pericarp sorghum, which ranged from 1.0 to 11.0 mg Eq. Lut.g^{-1}, similar to the values found in the present study.

In addition, Oliveira et al. (2017) studied the stability of anthocyanins and phenolic compounds content of sorghum genotype SC 319 flour and grain, in which there was no significant difference over 180 days of storage (4, 25, and 40 °C), presenting values from 0.40 to 0.43 mg Eq. Lut.g^{-1}. Such values are lower than those obtained in this work for the same genotype, corroborating research affirms that sorghum anthocyanins may be concentrated in the glumes and pericarp of the grain (Queiroz et al., 2014; Awika et al., 2005; Dykes et al., 2009). Also, according to Oliveira (2015), the total phenolic

contents showed no significant difference for grain and flour, with values ranging from 122.08 to 140.61 mg GAE.g^{-1}.

The decortication process of sorghum reduces the antioxidant activity and, consequently, the total phenolic compounds (Dlamini et al., 2007), which justifies the values obtained for the extract and powder dye. However, the content of phenolic compounds in the extract was higher than the values found for flours of 7 sorghum genotypes, which ranged from 0.26 to 4.5 mg GAE.g^{-1} (Queiroz et al., 2011).

The levels of anthocyanins and phenolic compounds in the powder dye were 1.85 higher than those found in the sorghum pericarp, indicating that, in addition to maintenance, these compounds were concentrated after drying. In a comparative study between the lyophilization process and conventional drying using hot air, Sablani et al. (2011) observed that freeze-drying provides greater retention of phytochemicals, which can increase the concentration of phenolic compounds, especially anthocyanins.

The colorimetric analysis of the sorghum pericarp extract and powder dye diluted in water (1:100) were presented in Table 5.

Table 5. Colorimetric evaluation of sorghum pericarp extract and diluted dye (1:100).

Parameters	Extract	Diluted dye
L*	40.97 ± 0.30 ^{b}	50.90 ± 0.12 ^{a}
a*	16.99 ± 0.96 ^{a}	7.04 ± 0.06 ^{b}
b*	31.42 ± 1.98 ^{a}	28.30 ± 0.15 ^{a}

*Means ± standard deviation followed by the same letter do not differ from each other by Tukey's test at 5% probability.

There was a significant difference between the initial extract and the diluted powder dye (1:100) for the L* and a* axes, indicating greater luminosity and lower reddish hue for the diluted powder dye. The total color difference (AE*) value calculated was equal to 15.42. Regarding the greater numerical difference for the parameter a*, Oliveira (2017) also reported such variation when comparing the sorghum flour extract and the grain during storage, in which the averages were 5.50 and 12.51, respectively. Thus, the intensity of the red color, characteristic of anthocyanin compounds, may have been influenced by the proportion of the mass of the dye in sorghum powder by the volume of dilution water used.

3.3 STABILITY OF THE POWDER DYE DURING STORAGE

There was no significant difference ($p>0.05$) for the levels of total phenolic compounds in the powder dye during storage (Table 6). The average obtained was 37.292

mg GAE.g^{-1}, higher than the phenolic compounds content of the original extract. Such a result was expected, Cardoso et al. (2014) reported that phenolic compounds of sorghum flour remained stable even after domestic processing with dry heat. Furthermore, the content of phenolic compounds in sorghum flour of genotype SC 319 remained stable for 180 days of storage at 25°C (Costa et al., 2016), a period even longer than that evaluated in the present study.

Table 6. Total anthocyanins and total phenolic compounds of the powder dye during storage.

Days of storage	**Total anthocyanins** (mg Eq. Lut.g^{-1})	**Total phenolic compounds** (mg GAE.g^{-1})
0	2.41 ± 0.02 ^{b}	36.80 ± 2.05 ^{a}
7	2.45 ± 0.10 ^{ab}	37.46 ± 1.36 ^{a}
14	2.55 ± 0.08 ^{a}	37.43 ± 1.05 ^{a}
21	2.25 ± 0.02 ^{c}	37.97 ± 3.01 ^{a}
30	2.38 ± 0.05 ^{bc}	37.59 ± 1.10 ^{a}

*Means ± standard deviation followed by the same letter, in columns, do not differ from each other by Tukey's test at 5% probability.

Evaluating the stability at room temperature, compared to day 0, there was an increase in anthocyanins content on the 14th day (2.553 mg Eq. Lut.g^{-1}) and a reduction on the 21st (2.247 mg Eq. Lut.g^{-1}). Antunes et al. (2017) evaluated the stability of lyophilized and vacuum-stored strawberry in which the lyophilization process did not influence the anthocyanin content, only after storage, with a significant increase until the 15th day and subsequent reduction on the 30th day.

However, the variations can be considered minimal and demonstrate that there are no important changes, emphasizing that on the last day of storage at room temperature, the total anthocyanin content of the powder dye did not differ significantly from day 0. This result corroborates previous studies that claim sorghum anthocyanins are more stable to pH and temperature variations, compared to those commonly found in vegetables and fruits (Awika et al., 2004; Dykes et al., 2009; Queiroz et al., 2014).

The study of colorimetric stability during storage is presented in Table 7.

Table 7. Colorimetric parameters during the storage at room temperature of the powder dye obtained from sorghum pericarp.

Days of storage	Parameters		
	L*	a*	b*
0	50.90 ± 0.12 [b]	7.04 ± 0.06 [e]	28.35 ± 0.15 [b]
7	49.33 ± 0.16 [c]	7.48 ± 0.05 [d]	27.82 ± 0.25 [b]
14	49.30 ± 0.09 [c]	8.32 ± 0.11 [c]	28.43 ± 0.23 [b]
21	53.41 ± 0.10 [a]	8.79 ± 0.20 [b]	31.82 ± 0.79 [a]
30	53.28 ± 0.25 [a]	9.11 ± 0.09 [a]	31.23 ± 0.50 [a]

*Means ± standard deviation followed by the same letter, in columns, do not differ from each other by Tukey's test at 5% probability.

There was an increase for luminosity (L*) in the last two days of analysis with values equal to 53.41 and 53.28. The same occurred with the parameter b* referring to the yellow hue, in which the values found for the 21st and 30th day were 31.82 and 31.23, respectively.

It was observed that the reddish hue (a*) increased during storage, showing a significant difference on all evaluated days. This fact is desirable since anthocyanins present the possibility of replacing the artificial dyes Red 40, Ponceau 4R, Erythrosine and Bordeaux S (Ozela et al., 2007).

3.4 GELATIN GUMMY

For total anthocyanins and total phenolic compounds in gelatin gummy elaborated with powder dye, the averages were 0.02 mg Eq. Lut.g^{-1} and 0.66 mg GAE.g^{-1}, respectively (Table 8). Values lower than the dye diluted in water (1:100), which may be related to the use of high temperature in the preparation of the candies, since the degradation of anthocyanin compounds increases with exposure to high temperature (Lopes et al., 2007). However, considering that a commercial gummy candy pack contains approximately 15 to 20 g, the use of dye extracted from sorghum can add value to this kind of product and may substitute the use of artificial dyes.

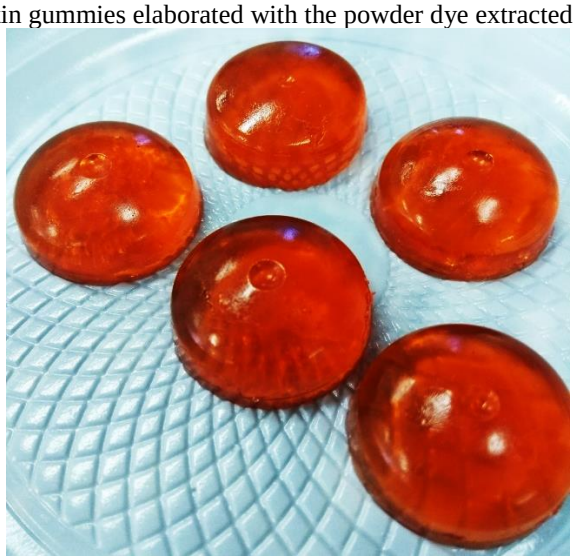
Table 8. Total anthocyanins, total phenolic compounds and colorimetric parameters of the gelatin candy made with powder dye from sorghum pericarp.

Parameters		Means ± standard deviation
Total anthocyanins (mg Eq. Lut.g^{-1})		0.02 ± 0,00
Total phenolic compounds (mg GAE.g^{-1})		0.66 ± 0.01
Color	L*	49.93 ± 0.67
	a*	28.90 ± 0.87
	b*	33.31 ± 0.89

The average obtained for the a* axis for the gelatin gummy was higher than that obtained for the powder dye, indicating an increase in the red hue. The values obtained

for the L* and b* axes were 49.93 and 33.31, respectively, giving lightness and yellow hue as shown in Figure 2.

Figure 2. Image of gelatin gummies elaborated with the powder dye extracted from sorghum pericarp.



The accentuated red hue is desirable since the acceptance of a food product by the consumer is directly related to its color, being essential in inducing the global sensation resulting from other characteristics such as aroma, flavor, and texture (Constant et al., 2002).

4 CONCLUSION

Sorghum pericarp of genotype SC 319 proved to be a good source of anthocyanin compounds, satisfactorily extracted using acidified water (3% citric acid) as a solvent, in the proportion 1:100. A reddish color powder was obtained, with stability of total anthocyanins and total phenolic compounds during 30 days of storage at room temperature. The application of the dye in the production of gelatin gummies gave color to the product despite the levels of anthocyanins and phenolic compounds being lower when compared to the powder dye. Therefore, the sorghum pericarp powder dye can be a viable and stable alternative to artificial dyes, and further research is relevant on its application conditions.

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