Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 14 (1)
January 2021
DOI: http://dx.doi.org/10.36560/141202111253
Article link
http://sea.ufr.edu.br/index.php?journal=SEA&page=article&op=view&path%5B%5D=1253&path%5B%5D=pdf
Included in DOAJ, AGRIS, Latindex, Journal TOCs, CORE, Discoursio Open Science, Science Gate, GFAR, CIARDRING, Academic Journals Database and NTHRYS Technologies, Portal de Periódicos CAPES, CrossRef, ICI Journals Master List.

ISSN 2316-9281

Microencapsulated *Myrciaria cauliflora* Berg. fruit peel extract as a natural pigment for neutralizing shampoo preparation

F. G. S. Oliveira[1], Y. C. Fernandes[1], F. G. S. Oliveira[2], J. Büttner-Pires[1], S. T. Cargnin[1], A. P. Sinhorin[1], J. R. G. S. Almeida[2], S. R. Ferrarini[1]

[1]Universidade Federal do Mato Grosso, Campus Sinos
[2] Universidade Federal do Vale do São Francisco, Campus Petrolina

*** Author for correspondence:** fernanda.gso@hotmail.com

Abstract. Hair is a complex natural fiber consisting mainly of keratin that can be divided into four main structural units, namely: cuticle, cortex, medulla, and membrane complex. The use of pigments in hair care preparations, mainly derivatives of aromatic amines, has been a topic of discussion due to toxicity and ecotoxicity concerns. In this background, *Myrciaria cauliflora* Berg is a native fruit occurring from northeastern to southern Brazil. Among the diversity of compounds detected in this fruit species are bioactive flavonoids and anthocyanins, which are responsible for the purple pigmentation of the fruit peels. This study reports on the use of a microencapsulated *Myrciaria cauliflora* Berg. peel extract as a temporary pigment to reduce the yellow tone of the hair. The extract was obtained by maceration with 70% ethanol (v/v) in an acidic milieu (6.0% citric acid). The extract was submitted to phytochemical profiling and a shampoo formulation was developed and applied onto a chemically treated hair. The preliminary characterization of the extract confirmed the presence of phenolic compounds and anthocyanins, in addition to the acid/base character of the extract. When tested on discolored hair samples with a yellow tone, the *Myrciaria cauliflora* Berg. shampoo reduced the yellow color, which was visually perceived by the enhancement of the hair brightness. These results demonstrate the potential of *M. cauliflora* extract – obtained from a fruit widely found in Brazil, as a temporary pigment to be used in cosmetic hair care.
Keywords: anthocyanins, *Myrciaria cauliflora*, shampoo, phytocosmetic

Introduction

Hair is a complex natural fiber consisting mainly of keratin that can be divided into four main structural units, namely: cuticle, cortex, medulla, and membrane complex. The cuticle, the outermost portion of a hair strand, is composed of amorphous protein material responsible for protecting the cortex from external aggression. The cortex is the middle part of the hair fiber, which constitutes about 70% of the hair mass and is the largest constituent of the fiber. The cortex is formed by aligned keratin microfibers organized in the direction of the strand, where melanin granules can be found. Interestingly, the type, size and quantity of melanin granules are key factors determining the hair color. The medulla is a structure forming the fiber core, consisting of specialized cells rich in glycogen and citronella. This structure corresponds to a small percentage of the capillary mass. Lastly, the membrane complex is a secondary component of the capillary fiber, composed mainly of lipids and proteins, which is responsible for joining adjacent cortical and cuticular cells (Peters et al. 2011; Hanumanthayya et al., 2018).

Mechanical damages and chemical treatments, such as discoloration of the hair strands, can cause changes in the chemical composition of the cuticle. Discoloration alters the natural melanin content in the cortex of the hair fiber, causes degradation of essential amino acids, and a number of other damages, which include increased porosity of the hair fibers and loss of brightness; in addition, the hair can be naturally damaged by solar radiation. Both prolonged exposure to radiation and the process of hair discoloration cause capillary oxidation, alter the melanin content and cause degradation of keratin, ultimately resulting in a yellowing hair (Peters et al. 2011; Nogueira et al., 2004).

The use of pigments in hair care preparations, mainly derivatives of aromatic amines, has been a topic of discussion due to toxicity and ecotoxicity concerns (Oliveira et al., 2014; Guerra-Tapia et al., 2014). Furthermore, common synthetic pigments such as anilines and gentian violet may dry

the hair strands, which reinforces the need for novel alternatives to these products. As naturally occurring products are readily available and widely used in cosmetology (Scotti et al., 2007), the exploration of their potential, in particular within the Brazilian biodiversity, has proven a promising possibility.

In this background, *Myrciaria cauliflora* Berg is a native fruit occurring from northeastern to southern Brazil (Sasso et al., 2010). Among the diversity of compounds detected in this fruit species are bioactive flavonoids and anthocyanins, which are responsible for the purple pigmentation of the fruit peels (Volp et al., 2008; Alves et al., 2013). In addition, the presence of free electrons in the nucleus and of free anthocyanins can be related to the antioxidant activity reported for these compounds (Baldin et al., 2016).

Both in discolored and natural hair, the fiber yellowing process takes place due to oxidative reactions. Hence, the antioxidant potential of anthocyanins and their effects in reducing the yellow hair tone warrant further investigation. As the stability and bioactivity of anthocyanins can be further increased with the use of microencapsulation technology (Baldin et al., 2016), we proposed in this pioneer study the development of a microencapsulated *M. cauliflora* peel extract formulation to neutralize the capillary yellow tone in discolored (with reflections, wicks and/or lights) hair.

Methods

Extract preparation

M. cauliflora fruits were collected in the countryside of Sinop, Mato Grosso, Brazil (11.80'.43'S and 55.57'43.2'W) between August and September 2016. Initially, the fruits were peeled manually to obtain the extract. Then, *M. cauliflora* peels were crushed in a knife mill and submitted to extraction with 70% ethanol (v/v) in acidic milieu (6.0% citric acid). This mixture was kept under refrigeration (4ºC) in the absence of light for 24 hours. The filtered extracts were concentrated to eliminate the solvent under reduced pressure at 40°C (rotary evaporator Buchi®R-3), remaining 30% of the initial volume. A portion of the extract material was concentrated and used for the characterization analysis while the remaining portion was used for microencapsulation.

Microencapsulation of M. cauliflora extract

The microencapsulation of *M. cauliflora* extract was performed using the lyophilization technique, as described elsewhere (Laine et al., 2008; Kechinski et al., 2010; Nogueira et al., 2017). To prepare the microencapsulated extract formulation, the wall material chosen (10% maltodextrin) was dissolved first, then the extract was added. The mixture was stirred for 3 hours and then submitted to freezing at -5ºC. The frozen material was lyophilized on a JJ Científica® equipment (Model LJJ02).

Total Phenol Content of M. cauliflora extract

The content of total phenolic compounds was established by the Folin-Ciocalteau method, modified by Shahidi & Naczk (1995). Briefly, samples were prepared with a concentration of 1 mg/mL in ultrapure water. The procedure was performed by adding 100 µL of the diluted extract into water, 500 µL of Folin-Ciocalteau (Merk®) reagent, and 6.0 mL of ultrapure water, in an amber glass. After 1 minute, 6.0 mL of a sodium carbonate solution (7.5 %) were added, stirred for a few seconds, and allowed to stand for 1 hour at room temperature. Lastly, the absorbance of the samples was read at 750 nm (Perkin Elmer®, UV / VIS Lambda 20). Gallic acid (Vetec®) was used as a reference standard and the curve was plotted with six concentrations ranging from 20 to 600 µg/mL (R^2 = 0.9987). The results were expressed as gallic acid equivalent per gram of extract (GAE/g).

Total monomeric anthocyanins content

The analysis of total monomeric anthocyanins content was performed according to the pH differential method described by Terci & Rossi (2002). An aliquot of 3.0 mL of the lyophilized material at 1 mg/mL was diluted into 10 mL of an acidified ethanol solution (pH 1.0 and 4.5). Absorbance readings were performed at wavelengths ranging from 517 nm to 700 nm (Perkin Elmer®, UV / VIS Lambda 20). The same acidified ethanol solution was used as a blank solution. The concentration of anthocyanins was calculated as cyanidin-3-glycoside (M = 449.2 g.mol-¹) from the equations I (A = (A_{517}-A_{700}) pH 1.0 - (A_{517}-A_{700}) pH 4.5) and II (C = A.Mm / Ɛ.b, where Ɛ = 26,900 L / mol.cm and b = 1.0).

Characterization reactions

In aqueous solutions, anthocyanins can present different structures depending on the pH. Due to their acid/base character, the color of these compounds may change and therefore can be used as a pH indicator (Terci & Rossi, 2002). pH indicators are weakly acid (acid indicators) or weakly basic (basic indicators) organic substances that present different colors for their protonated and deprotonated forms. The color variation occurs when the hydrogen ion (acid) is added to, or removed from, the molecule. Anthocyanins may produce blue, violet, red and pink coloration of flowers and fruits (Kong et al., 2003). To verify the acid-base character of the *M. cauliflora* peel extract, the concentrated extract was added to citrate-phosphate buffer solutions at pH 2.0, 5.0, 7.0, and 9.0.

Ferric Chloride Reaction and Cyanidin Reaction or Shinoda Assay

To determine the presence of polyphenols in the *M. cauliflora* extract, two drops of 2.5% (p/v) ferric chloride were added to 1.0 mL of *M. cauliflora* peel extract. In the presence of ferric chloride, the extract may develop coloration varying between green, yellow-brown and blue-violet depending on the type of compound present in the sample (Li et al., 2018).

For the cyanidin reaction, 3.0 mL of *M. cauliflora* peel extract were used and oven-heated at

80°C until complete drying. Thereafter, 1.5 ml of methanol, magnesium (1.0 g) and 0.5 mL of concentrated HCl, were added.

Antioxidant activity by DPPH assay

The quantitative evaluation of the antioxidant activity of *M. cauliflora* extract against the stable DPPH radical was performed by adding 0.0237 mg of DPPH into 1.0 ml of the methanolic solution of *M. cauliflora* extract at different concentrations (25, 50 and 100 µg/mL). The decrease in absorbance was evaluated after 30 minutes of reaction at λ = 517 nm (Perkin Elmer®, UV / VIS Lambda 20) (Kordali et al., 2005; Sharma et al., 2009; Ratz-Lyko et al., 2012). Methanol was used as a blank solution and a 25% (p/v) DPPH solution was used as a control. Hydroxytoluene-butylated (BHT) was used as a reference antioxidant compound. A sample of the shampoo containing 10% (w/w) of the microencapsulated *M. cauliflora* peel extract (see topic 2.8 for further details) was also evaluated at the concentrations mentioned above. In such a case, the base shampoo was used as a control. The experiments were performed in triplicate and the antioxidant activity was calculated according to the equation: % of DPPH radical scavenging = [(control absorbance - sample absorbance) / (control absorbance)] × 100.

Shampoo development

A shampoo containing the microencapsulated *M. cauliflora* peel extract was prepared. For the development of the shampoo base (SH_0) for normal hair, an aqueous solution containing 25% sodium lauryl ether sulphate was initially prepared. Then 5% triethanolamine lauryl sulphate, 3% fatty acid diethanolamine and 1% methyl paraben were added to the solution and homogenized slowly. The pH was adjusted to 5.5 using a 20% citric acid solution and the viscosity was obtained with a solution of 20% sodium chloride. The shampoo samples containing the microencapsulated *M. cauliflora* peel extract were prepared at the concentrations of 1.0, 5.0 and 10.0% ($SH_{1.0}$, $SH_{5.0}$, $SH_{10.0}$). Additionally, a shampoo sample containing 0.1 % BHT, a synthetic antioxidant agent, was prepared.

Preliminary stability

Preliminary stability assays were performed in triplicate, which included macroscopic analysis, pH tests, thermal stress, centrifugation test, and freeze-thaw cycles. The shampoo samples (SH_0, $SH_{1.0}$, $SH_{5.0}$, $SH_{10.0}$ and SH_{BHT}) were stored at room temperature and analyzed at day 0 (24 hours post-formulation) and after the freeze-thaw cycle (Tozetto et al., 2017).

Macroscopic analysis, Thermal stress, Freeze-thaw cycle and Centrifuge test

The macroscopic characteristics of the formulations were determined based on the following parameters: appearance, color, odor and homogeneity.

For the thermal stress testing, the samples were submitted to gradual heating in a water bath (Quimis® - Model Q334M-28) until reaching a temperature of 80ºC, which was maintained for 30 minutes (22).

For the freeze-thaw cycle, the samples were submitted to freezing conditions at -5 ± 2ºC for 24 hours and then incubated at 40 ± 2ºC for 24 hours, thus completing one cycle. Twelve freeze-thaw cycles were performed, totaling twelve days of testing (Brazil, 2004).

For the centrifuge test, 5.0 g of each formulation (kept at room temperature after thermal stress and the freeze-thaw cycle) were added to a conical test tube and subjected to centrifugation (Fanem-Excelsa II - Model 206 BL) with cycles of 1000, 2500 and 3500 rpm for 15 minutes at room temperature (23).

Determination of pH and Viscosity

The pH of the *M. cauliflora* extract and shampoo samples was determined by dilution in distilled water (1:20 w/v) at 25 °C. The pHmeter (Tecnopon-Model mPA210) was calibrated with known pH standard solutions (4.0, 7.0 and 10.0), and pH readings were performed in triplicate (Brazil, 2004).

Ford cup viscosity analysis were performed at 25 °C, with the hole number 4 in the Ford cup closed with the finger and filled with the samples. The orifice was released, and the chronometer was started simultaneously. When the first flow interruption occurred, the chronometer was stopped, and the time spent was then recorded. The calculation was performed according to the hole used in the measurement to obtain the viscosity in the centipoise unit (cP).

Accelerated Stability

A sample of the shampoo containing the microencapsulated *M. cauliflora* extract at the highest concentration (SH_{10}) was tested for the accelerated stability analysis. Test (SH_{10}) and control formulations (SH_0 and SH_{BHT}) were submitted to three different temperature conditions, as follows: 4 ± 2ºC (Compact Consul Refrigerator 120 liters), 25 ± 2ºC (controlled room temperature) and 45 ± 2ºC (New Ethics Climate 420 - CLD 300) for a period of 90 days (22). Macroscopic, pH and viscosity analyses were performed at days 0, 30, 60 and 90.

Hair sample analysis

Samples of discolored hair with reflections, wicks and/or lights, with yellow color tone, were kindly donated by beauty salons. The different types of hair were treated with the shampoo (1.5 g) containing the microencapsulated M. cauliflora peel extract [10% (w/w)] ($SH_{10.0}$). In each sample, a manual process was performed simulating a capillary wash. After 3 minutes, the samples were washed twice with water to remove the product. The hair samples were dried with a hairdryer and analyzed by visual comparison to an untreated hair.

Results and discussion

Extract preparation and total phenol content of M. cauliflora extract

To evaluate the chemical composition on *M. cauliflora,* the peels were extracted by the maceration method using 70% ethanol (v/v) and the yield of 45.8% was obtained, resulting in an appropriate extraction. The total phenolic content of *M. cauliflora* peel extract was 580.41 mg GAE/g of extract.

Total monomeric anthocyanins content

In the analysis of total anthocyanins, the *M. cauliflora* extract had a cyanidin concentration of 11.5 mg/ml, which was the major anthocyanin detected in the extract.

Characterization reactions

The ethanolic extract of *M. cauliflora* fruit peels presented blue color when in contact with 10% sodium hydroxide, therefore indicating the presence of anthocyanins therein. As anthocyanins present different structures based on the pH of the solution, the extract was submitted to differential pH analysis. The *M. cauliflora* extract presented different staining according to the pH tested, as shown in Figure 1. In acidic milieu (pH 2.0), the anthocyanins presented red color, since there is a predominance of the flavilic cation form. When the pH was adjusted to 5.0 the staining reduced considerably, with a predominance of the pseudocarbinol form. At pH higher than 6.0, there was a disruption of the heterocyclic ring. Therefore, the staining promoted by the different types of anthocyanins varied based on the pH of the solution (Marco et al., 2008).

Ferric chloride reaction and cyanidin reaction or Shinoda assay

When ferric chloride was added to the diluted sample, a visible violet coloration was observed, thereby indicating the presence of polyphenols in the extract, especially flavonoids and hydrolysable tannins.

The reaction of cyanidin was developed by the action of magnesium in the presence of concentrated HCl and *M. cauliflora* extract. A blood red staining (Figure 2) was observed, indicating the presence of flavonols or dihydroflavonol in the extract. The presence of different compounds leads to different colors.

Antioxidant activity by DPPH assay

The *M. cauliflora* extract showed a dose-response curve showing an increase in potential antioxidant activity as the extract concentration increased, a characteristic observed even after incorporation of the extract into the shampoo base (Table 1). The *M. cauliflora* extract and BHT at the concentration of 100 µg/mL produced a percentage of inhibition of DPPH around 90%, indicating that the natural product presented antioxidant activity equivalent to that of the synthetic compound.

Shampoo development and preliminary stability

The macroscopic analysis demonstrated that the experimental shampoos (SH_0, $SH_{1.0}$, $SH_{5.0}$, $SH_{10.0}$ and SH_{BHT}) were transparent, homogeneous and viscous at room temperature. The bases containing the extract of *M. cauliflora* showed characteristic color, which increased as a function of the extract concentration. The formulations demonstrated stability even under thermal stress since these characteristics were maintained throughout the experimental period.

The formulations tested showed antioxidant activity (Table 3). The formulation $SH_{10.0}$ at the concentration of 100 µg/mL showed a percentage of inhibition of DPPH of 84,24 ± 3,20.

None of the experimental shampoo formulations showed any physical instability, such as phase separation (coalescence, cremation or flocculation) after centrifugation, as well as thermal stress or freeze-thaw cycles followed by centrifugation throughout the duration of the study.

A pH analysis of the ethanolic extract and shampoo samples was also carried out. The findings at day 0 and after the freeze-thaw cycle showed no significant difference in pH values at day zero even after thermal stress (Table 2). This result implies in the stability of the formulation containing or not *M. cauliflora* extract at different concentrations. It is also noted that increasing the concentration of extract in the shampoos did not modify the pH of the formulations. However, there was a significant increase in the pH of the shampoo without *M. cauliflora* extract after 12 days of the feeze-thaw cycle. The initial pH of the formulation was 5.88 and after the stability study, it increased to 7.16. This phenomenon could be attributed to the loss of citric acid activity or interaction between the components of the formulation. It is worth noting, therefore, that the presence of *M. cauliflora* extract provided physical-chemical stability to the system, keeping the pH close to baseline levels.

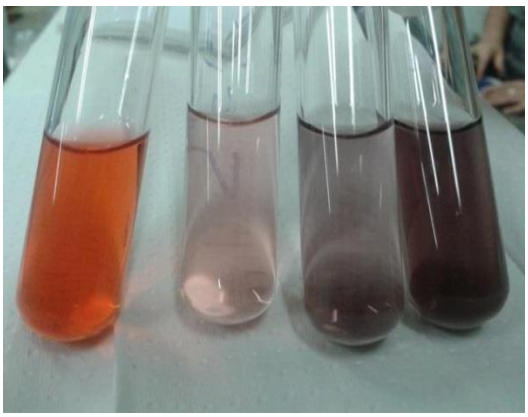

Figure 1. Characterization reactions of anthocyanins from the extract at different pHs (2.0, 5.0; 7.0 and 9.0 from left to right).

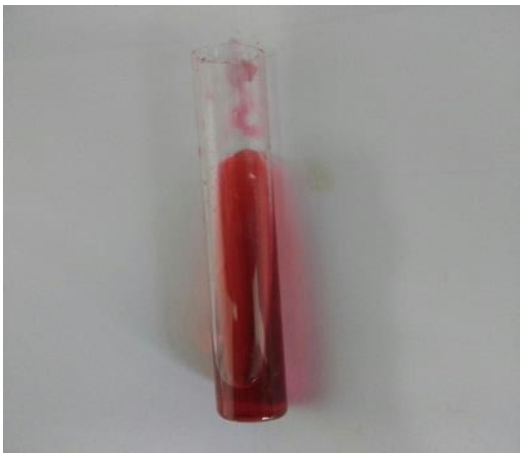

Figure 2. Cyanidin Reaction or Shinoda Assay.

Table 1. Antioxidant activity of the microencapsulated *M. cauliflora* extract and standard synthetic antioxidants.

Samples	% of DPPH radical scavenging
Microencapsulated *M. cauliflora* peel extract	81,61 ± 2,79[a]
SH_{100}	84,24 ± 3,20[a]
BHT	91,82 ± 0,92[b]

Table 2. pH analysis of preliminary stability of the formulation at day 0 and after the freeze-thaw cycle (n=3)

	Day zero		After freeze-thaw cycle
Sample	**pH***	**pH****	**pH***
SH_0	5.60± 0.03	5.66± 0.05	5.88 ± 0.04
SH_{BHT}	5.70± 0.03	5.63 ± 0.05	5.80± 0.03
$SH_{1.0}$	5.55± 0.02	5.55 ± 0.03	5.55± 0.05
$SH_{5.0}$	5.60± 0.04	5.66± 0.01	5.70± 0.01
$SH_{10.0}$	5.55± 0.02	5.60± 0.05	5.55± 0.03

*pH before thermal stress; **pH after thermal stress.

Accelerated Stability

The SH_0 and SH_{BHT} formulations did not show macroscopic changes in color and odor as a function of time, while shampoos containing the extract ($SH_{10.0}$) presented constant purple coloration. This is a desired situation for a formulation that exhibits anti-yellowing activity for the hair. Figure 3 shows the pH variation of the samples as a function of time and environmental conditions. The SH_0 and SH_{BHT} formulations maintained at 4 ± 2°C had an increased pH after 30 days of study, remaining unchanged up to 90 days. The $SH_{10.0}$ formulation presented a slight decrease in this parameter; however, with no significant difference between the days analyzed. The formulations maintained at 45 ± 2°C, SH_{BHT} and $SH_{10.0}$, presented a decrease in pH after 30 days of study, which was reverted 60 days following the initial tests.

The viscosity analysis showed that all samples kept in refrigerator remained unaltered regarding this parameter (Figure 4). On the other hand, the base shampoo maintained at 25 ± 2°C and 45 ± 2°C showed significant reduction in viscosity after 30 days of analysis. At room temperature, this

change was reversible over time. However, this characteristic was irreversible when the sample was stored at a higher temperature.

Hair sample analysis

Discolored hair samples were treated with the base shampoo containing 10% microencapsulated *M. cauliflora* extract, and then dried with a hairdryer. The hair strands samples with lights/wicks showed excellent results in color neutralization. We observed that the yellowish tone was neutralized and showed a clear natural color (Figure 5).

The chemical composition and content of metabolites in plant extracts is variable and depends on the fruit species and extraction method. The extraction of *M. cauliflora* peels by the maceration method using 70% ethanol (v/v) was shown to be more effective than those reported by Santos, Veggi, Meireles (2012) (Santos et al., 2012). These authors used ethanol as extracting agent under ultrasound, continuous agitation and Soxhlet, resulting in yields ranging from 9 to 12 % (p/v). On the other hand, the extract obtained in our study resulted in a low viscosity liquid, showing a dark red to purple color, with characteristic odor and pH, consistent with that obtained by Nazaré, et al. (2002).

The development of a more stable extract through microencapsulation allows for its use in the food, pharmaceutical, and cosmetic industry. Kechinski (2010) concluded that, as compared to carboxymethylcellulose and hydroxymethylpropylcellulose, maltodextrin at 10% is the best encapsulating polymer used for microencapsulation of anthocyanins. Maltodextrin is a low-cost polysaccharide obtained by the acid hydrolysis of starches, for instance, potatoes and others, and it does not influence the flavor and final aroma of the formulations, which makes it an interesting encapsulation polymer. Therefore, 10% maltodextrin was chosen as the encapsulation agent for the microencapsulation of *M. cauliflora* peel extract. Importantly, the microencapsulation technique preserves the color of the pigments, reducing oxidation and maintaining the quality of the formulation for long periods of time.

The total phenol amount observed herein was higher than that found by Hamm (2009) (499.10 mg GAE/g of extract) and Silva (2011) (435.38 mg GAE/g of extract). The extraction process along with lyophilization and the use of maltodextrin as the encapsulation agent may have possibly contributed to retaining the phenolic compounds more efficiently.

Characterization reactions were developed for the phtyochemical screening, and *M. cauliflora* peels were found to have two major anthocyanins, delphinidin (3-O-glucoside) and cyanidin (cyanidin-3-glucoside), in which cyanidin concentration is four times higher than that of delphinidin (Wu et al., 2012). The cyanidin amount observed in our study was higher than that reported by Santos et al. (2010) for all extracts obtained (< 7 mg cyanidin-3-glycoside/g dry material). Possibly, the slightly acidified extractive solvent (70% ethanol, acidified with 6.0% citric acid) may have facilitated the release and solubilization of anthocyanins from the fruit peels (Baldin et al., 2010). Thus, the extraction method used herein proved to effectively extract anthocyanins from *M. cauliflora* fruit peels.

For instance, when flavones are present in the sample, the yellow coloration turns to red, flavonols lead to a red coloration, while flavonones turn the solution from red to purple; anthocyanin derivatives turn to red, becoming pink; and chalcones, aurones, dihydrochalcones, isoflavones and isoflavanones do not react, presenting no coloration (Pedroso et al., 2009).

For the DPPH assay, the result found in this study was superior to that found in a study performed by Hacke et al. (2016), who observed 82.79% of the antioxidant potential. There was no significant difference between the results obtained with the extract before and after incorporation into the shampoo. The shampoo base and BHT did not influence the test, demonstrating that the extract presents an excellent antioxidant potential and can therefore be incorporated into shampoos for this purpose. Therefore, the *M. cauliflora* extract constitutes a great alternative for use as an antioxidant agent in cosmetic formulations.

The evaluation of formulation stability is commonly accomplished through methods that accelerate instability, in which the formulation is subjected to stressing conditions such as thermal variation, exposure to light, among others (Montagner et al., 2004). Based on this, the thermal stress test was developed in order to accelerate the physical-chemical interactions between the raw materials by increasing the temperature in a short period of time (Rieger, 1996). In our study, we observed that as the temperature increased, the viscosity of the formulation decreased, although this expected characteristic was found to be reversible when samples returned to room temperature. No signs of phase separation, release of gases and/or color changes were observed. Another effective method for determining the physical stability of cosmetic products is the centrifuge test. In this test, a change in the gravity force occurs, thereby accelerating the physical processes of sedimentation, cremation or coalescence of the system through phase separation. The pH analysis also showed results within the requirements of legislation. The results found in this study suggest these shampoo formulations are physically stable, since there were no macroscopic changes in color and odor after all the stability assays.

The hair sample assay showed that the yellowish tone was neutralized. The main capillary structure related to the response to such a procedure is the cuticle. Agents acting on this structure are responsible for malleability, softness and combability, and mainly for hair shine (Souza & Antunes-Jr, 2009). Based on the findings of this study, we can infer that the daily use of capillary preparations containing

anthocyanins as a natural pigment in the formulation, in discolored hairs with lights/wicks, positively affect the process of removing the yellow color of the hair, which is mainly observed by the increase in capillary brightness.

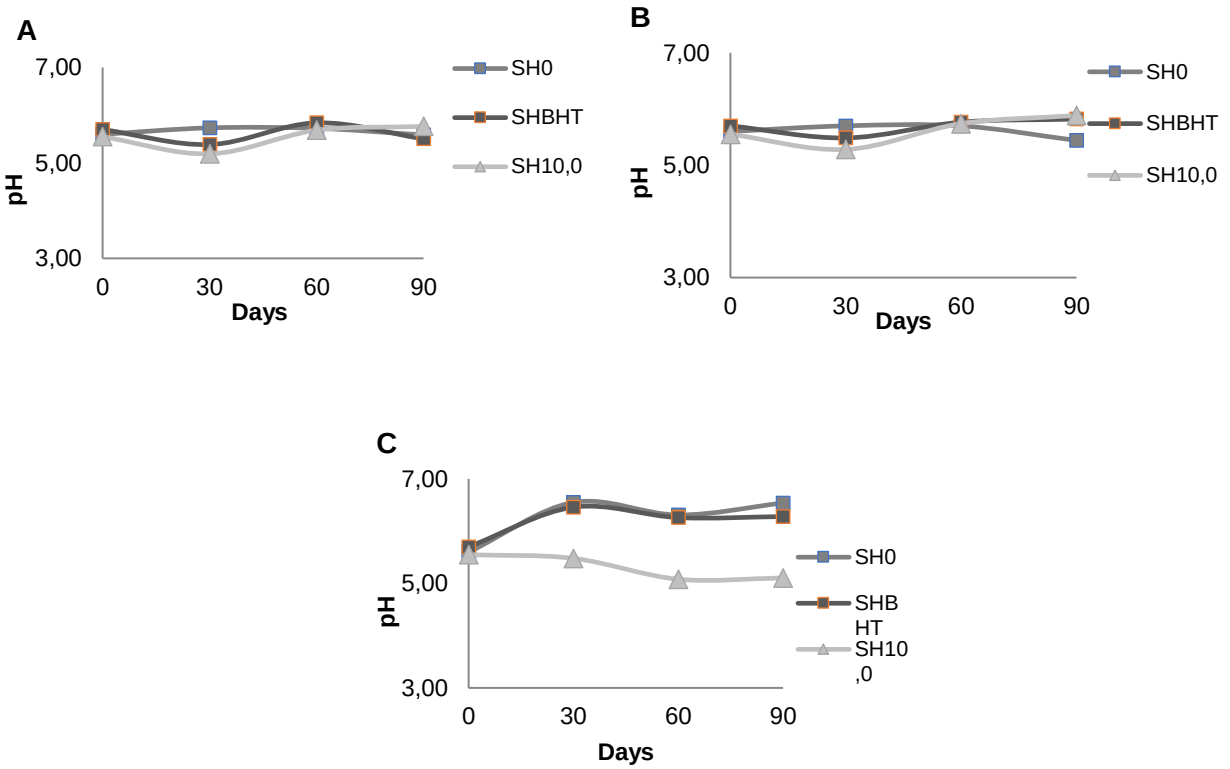

Figure 3. pH analysis: A) 4 ± 2ºC; B) 25 ± 2ºC and C) 45 ± 2ºC

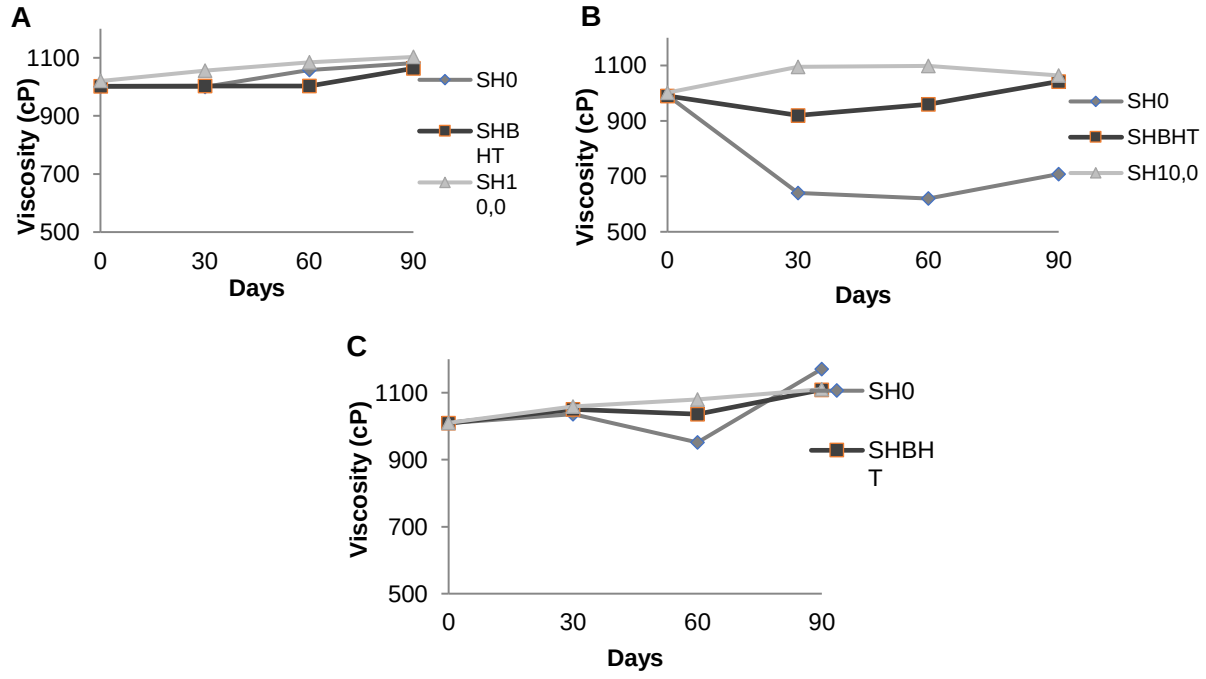

Figure 4. Viscosity analysis: A) 4 ± 2ºC; B) 25 ± 2ºC and C) 45 ± 2 ºC

Figure 5. Whitened hair: A) before cleansing; B) hair washed with the experimental shampoo formulation.

The ethanolic extract of *M. cauliflora* peel was found to possess a high content of phenolic compounds and anthocyanins. The latter present different structures as a function of different pH values, which makes them potentially useful natural pH indicators. In addition, the DPPH assay showed that the antioxidant potential of the extract was close to the synthetic antioxidant and that the shampoo base did not influence the antioxidant capacity of these compounds upon incorporation.

The shampoo samples containing *M. cauliflora* extract were stable even under adverse conditions. In the treatment of hair with yellowish coloration, the formulation containing 10% microencapsulated ethanolic extract of *M. cauliflora* presented a positive result, neutralizing the yellowing process. Taken altogether, these findings indicate the potential use of anthocyanins in shampoo formulations aimed at treating hair yellowing. Thus, *M. cauliflora*, an abundant and low-cost Brazilian fruit mainly composed of anthocyanins in its peels, demonstrated promising antioxidant and hair-toner characteristics. The potential of this fruit for cosmetic purposes, especially cosmetic hair care, warrants further investigation.

Conclusion

Taken altogether, these findings indicate the potential use of anthocyanins in shampoo formulations aimed at treating hair yellowing. Thus, *M. cauliflora*, an abundant and low-cost Brazilian fruit mainly composed of anthocyanins in its peels, demonstrated promising antioxidant and hair-toner characteristics. The potential of this fruit for cosmetic purposes, especially cosmetic hair care, warrants further investigation.

Acknowledgment

The authors thank the Federal University of Mato Grosso (UFMT - Campus Sinop) for financial support.

References

ALVES, A.P.C.; CORRÊA, A.D.; PINHEIRO, A.C.M.; OLIVEIRA, F.C. Flour and anthocyanin extracts of jaboticaba skins used as a natural dye in yogurt. International Journal of Food Science and Technology 48: 2007-2013, 2013. https://doi.org/10.1111/ijfs.12110

BALDIN, J.C.; MICHELIN, E.C.; POLIZER, Y.J.; RODRIGUES, I.; DE GODOY, S.H.; FREGONESI, R.P.; PIRES, M.A.; CARVALHO, L.T.; FÁVARO-TRINDADE, C.S.; DE LIMA, C.G.; FERNANDES, A.M.; TRINDADE, MA. Microencapsulated jabuticaba (*Myrciaria cauliflora*) extract added to fresh sausage as natural dye with antioxidant and antimicrobial activity. Meat Science 118:15-21, 2016. https://doi.org/10.1016/j.meatsci.2016.03.016

BRASIL. Agência Nacional de Vigilância Sanitária. Guia de estabilidade de produtos cosméticos. 1ª ed. Brasília: ANVISA, 2004. http://portal.anvisa.gov.br/documents/106351/107910/Guia+de+Estabilidade+de+Produtos+Cosm%C3%A9ticos/49cdf34c-b697-4af3-8647-dcb600f753e2

GUERRA-TAPIA, A.; GONZALEZ-GUERRA, E. Hair cosmetics: Dyes. Actas Dermo-Sifiliogr 105:833-839, 2014. https://doi.org/10.1016/j.adengl.2014.02.003

HACKE, A.C.M.; GRANATO, D.; MACIEL, L.G.; WEINERT, P.L.; PRADO-SILVA, L.D.; ALVARENGA, V.O.; DE SOUZA SANT'ANA, A.; BATAGLION, G.A.; EBERLIN, M.N.; ROSSO, N.N. Jabuticaba (*Myrciaria cauliflora*) Seeds: Chemical characterization and extraction of antioxidant and antimicrobial compounds. Journal of Food Science 81:C2206-2217, 2016. https://doi.org/10.1111/1750-3841.13405

HAMM, J.H.G.; MANICA-BERTO, R.; CONTREIRA, C.L.; PEGORARO, C.; RUFATO, A.R.; SILVA, J.A. Estudo fitoquímico em frutos da família Myrtaceae. Pelotas: Editora e Gráfica Universitária, 2009.

HANUMANTHAYYA, K.; BALASUBRAHMANYAM, G.; MENAKAA, M.; SUNITHA, M.; NIKHIL, N. Hair Coloring – Hair Dyeing. Journal of Medical Science 38:13-17, 2018.

KECHINSKI, C.P.; GUIMARÃES, P.V.R.; NOREÑA, C.P.Z.; TESSARO, I.C.; MARCZAK, L.D.F. Degradation Kinetics of Anthocyanin in Blueberry Juice during Thermal Treatment. Journal of Food Science 75:C173-176, 2010. https://doi.org/10.1111/j.1750-3841.2009.01479.x

KONG, J.M.; CHIA, L.S.; GOH, N.K.; CHIA, T.F. Analysis and biological activities of anthocyanins. Phytochemistry 64:923-933., 2003. https://doi.org/10.1016/S0031-9422(03)00438-2

KORDALI, S.; CAKIR, A.; MAVI, A.; KILIC, H.; YILDIRIM, A. Screennig of chemical composition and antifungal activity of essential oils from three Turkish *Artemisia* species. Journal of Agricultural and Food Chemistry 53:1408–1416, 2005. https://doi.org/10.1021/jf048429n

LAINE, P.; KYLLI, P.; HEINONEN, M.; JOUPPILA, K.

Storage stability of microencapsulated cloudberry (*Rubus chamaemorus*) phenolics. Journal of Agricultural and Food Chemistry. 56:11251-11261, 2008. https://doi.org/10.1021/jf801868h

LI, L.; ZHANG, M.; ZHANG, S.; CUI, S.; SUN, B. Preparation and antioxidant activity of ethyl-linked anthocyanin-flavanol pigments from model wine solutions. Molecules 23:1066, 2018. https://doi.org/10.3390/molecules23051066

MARCO, P.H.; POPPI, R.J.; SCARMÍNIO, I.S. Procedimentos analíticos para identificação de antocianinas presentes em extratos naturais. Química Nova 31:1218-1223, 2008. https://doi.org/10.1590/S0100-40422008000500051

MONTAGNER, D.; CORRÊA, G.M. Avaliação da estabilidade de cremes com ureia em diferentes pHs. Revista Brasileira de Farmácia 85:69-72, 2004. https://www.rbfarma.org.br/files/pag_69a72.pdf

NAZARÉ, R.F.R.; DE OLIVEIRA, M.S.P.; DE CARVALHO, J.E.U. Avaliação de progênies de açaizeiro como fonte de corantes naturais para alimentos. In: Congresso Brasileiro de Fruticultura, 2002. https://core.ac.uk/display/45494997

NOGUEIRA, A.C.S.; JOEKES, I. Hair color changes and protein damage caused by ultraviolet radiation. Journal of Photochemistry and Photobiology B 74:109-111, 2004. https://doi.org/10.1016/j.jphotobiol.2004.03.001

NOGUEIRA, M.B.; PRESTES, C.F.; BURKERT, J.F.M. Microencapsulation by lyophilization of carotenoids produced by *Phaffia rhodozyma* with soy protein as the encapsulating agent. Food Science and Technology 37:1-4, 2017. https://doi.org/10.1590/1678-457x.05417

OLIVEIRA, R.A.G.; ZANONI, T.B.; BESSEGATO, G.G.; OLIVEIRA, D.P.; UMBUZEIRO, G.A.; ZANONI, M.V.B. The chemistry and toxicity of hair dyes. Química Nova 37:1037-1046, 2014. https://doi.org/10.5935/0100-4042.20140143

PEDROSO, R.; SILVA, C.D.P.; FURLAN, C.M. Comparison of major chemicals constituents in two species of arnica: cravorana (*Porophyllum ruderale* [jacq.] Cass) e varão-de-ouro (*Solidago* sp.). Revista Brasileira de Ciências da Saúde. 7:42-49, 2009.

PETERS, E.M.; IMFELD, D.; GRÄUB, R. Graying of the human hair follicle. Journal of Cosmetic Science 62:121-125, 2011.

PIANOVSKI, A.R.; VILELA, A.F.G.; DA SILVA, A.A.S.; LIMA, C.G.; DA SILVA, K.K.; CARVALHO, V.F.M.; MUSIS, C.R.; MACHADO, S.R.P.; FERRARI, M. Use of pequi oil (*Caryocar brasiliense*) in cosmetics emulsions: development and evaluate of physical stability. Brazilian Journal of Pharmaceutical Sciences 44:251-259, 2009. https://doi.org/10.1590/S1516-93322008000200010

RATZ-LYKO, A.; ARCT, J.; PYTKOWSKA, K. Methods for evaluation of cosmetic antioxidant capacity. Skin Research and Technology 18:421–430, 2012. https://doi.org/10.1111/j.1600-0846.2011.00588.x

RIEGER, M.M. Teste de estabilidade para macroemulsões. Cosmetics & Toiletries 8:47-53, 1996.

SANTOS, D.T.; VEGGI, P.C.; MEIRELES, M.A.A. Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: Yield, composition and economical evaluation. Journal of Food Engineering 01:23–31, 2010. https://doi.org/10.1016/j.jfoodeng.2010.06.005

SANTOS, D.T.; VEGGI, P.C.; MEIRELES, M.A.A. Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins. Journal of Food Engineering 108:444–452, 2012. https://doi.org/10.1016/j.jfoodeng.2011.08.022

SASSO, S.A.Z.; CITADIN, I.; DANNER, M.A. Cutting propagation of jabuticaba tree. Revista Brasileira de Fruticultura 32:577-583, 2010. https://doi.org/10.1590/S0100-29452010005000054

SCOTTI, L.; SCOTTI, M.T.; CARDOSO, C.; PAULETTI, P.; CASTRO-GAMBOA, I.; BOLZANI, V.D.S.; VELASCO, M.V.R.; MENEZES, C.M.S.; FERREIRA, E.I. Molecular modeling applied to the development of molecules with antioxidant activity for cosmetic use. Brazilian Journal of Pharmaceutical Sciences 43:153-166, 2007. https://doi.org/10.1590/S1516-93322007000200002

SHAHIDI, F.; NACZK, M. Food phenolics: sources, chemistry, effects and applications. Food Chemistry 57:481-482, 1995.

SHARMA, O.P.; BHAT, T.K. DPPH antioxidant assay revisited. Food Chemistry 113:1202–1205, 2009. https://doi.org/10.1016/j.foodchem.2008.08.008

SILVA, P.I. Otimização da extração e microencapsulamento de fenólicos e antocianinas de jabuticaba (*Myrciaria jabuticaba*). 80 f. (Dissertation) - Universidade Federal de Viçosa, Viçosa, Brazil, 2011.

SOUZA, V.M.; ANTUNES-JR, D. Ativos dermatológicos: guia de ativos dermatológicos utilizados na farmácia de manipulação para médicos e farmacêuticos. São Paulo: Tecnopress, 2009.

TERCI, D.B.L.; ROSSI, A.V. Indicadores naturais de pH: usar papel ou solução? Química Nova 25:684-688, 2002. https://doi.org/10.1590/S0100-40422002000400026

TOZETTO, J.T.; TOZETTO, A.T.; HOSHINO, B.T.; ANDRIGHETTI, C.R.; RIBEIRO, E.B.; CAVALHEIRO, L.; FERRARINI, S.R. Extract of *Punica granatum* L.: an alternative to BHT as an antioxidant in semissolid emulsified systems. Química Nova 40:97-104, 2017. https://doi.org/10.21577/0100-4042.20160160

VOLP, A.C.P.; RENHE, I.R.T.; BARRA, K.; STRINGUETA, P.C. Flavonoídes anthocyanins: Características e propriedades na nutrição e saúde. Revista Brasileira de Nutrição Clínica 23:141-149, 2008.

WU, S.B.; DASTMALCHI, K.; LONG, C.; KENNELLY, E.J. Metabolite profiling of jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. Journal of Agricultural and Food Chemistry 60:7513–7525, 2012. https://doi.org/10.1021/jf301888