Food Chemistry 245 (2018) 966–973

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Stabilizing effect of montmorillonite on acerola juice anthocyanins

Hálisson L. Ribeiro[a], Ana Vitória de Oliveira[a], Edy S. de Brito[b], Paulo R.V. Ribeiro[b], Men de sá M. Souza Filho[b], Henriette M.C. Azeredo[b,c,*]

[a] Federal University of Ceara, Campus Pici, Bloco 709, 60455-760 Fortaleza, CE, Brazil
[b] Embrapa Agroindústria Tropical, R. Dra. Sara Mesquita, 2270, Pici, 60511-110 Fortaleza, CE, Brazil
[c] Embrapa Instrumentação, R. 15 de Novembro, 1452, 13560-970 São Carlos, SP, Brazil

ARTICLE INFO

Keywords:
Clays
Silicates
Cation exchange
Natural pigments

ABSTRACT

This study was conducted to evaluate color and anthocyanin stability of clarified acerola juice (CAJ) as affected by montmorillonite (Mnt) at different concentrations (0–6 wt%, dry basis). While non-complexed CAJ suffered noticeable color degradation with time and pH variations, the presence of Mnt (especially at 4–6 wt%) not only changed the initial color of CAJ but also made it more stable with time and pH changes. CAJ/Mnt mixtures were ultracentrifuged in order to separate them into supernatants and anthocyanin-complexed Mnt precipitates. The supernatants presented decreasing anthocyanin contents with increasing Mnt concentrations, indicating pigment retention by the precipitates. X-ray diffraction of precipitates showed that Mnt interlayer spacing was increased by increasing anthocyanin/Mnt ratios, corroborating anthocyanin intercalation. FTIR revealed a band at 1530 cm^{-1} ascribed to formation of anthocyanin-Mnt complexes. Moreover, chromatograms indicated the selective adsorption of two compounds by Mnt, which were identified by LC-MS as cyanidin-3-*O*-rhamnoside and pelargonidin-3-*O*-rhamnoside.

1. Introduction

Acerolas (*Malpighia emarginata*), also known as Barbados cherries, have been increasingly produced, because of their high vitamin C contents (from 700 to 1400 mg/100 g^{-1}, according to Cunha Neto, Rabelo, Bertini, Marques, & Miranda, 2012). They also contain anthocyanins in contents ranging from 3.8 to 47.4 mg/100 g of fruit pulp, depending on the cultivar (Musser et al., 2004), the main anthocyanins being cyanidin-3-rhamnoside and pelargonidin-3-rhamnoside (Brito et al., 2007; De Rosso et al., 2008). Acerolas are mostly processed into pasteurized juices and frozen purees rather than consumed fresh, because of their high perishability and high acidity (SEBRAE, 2016). However, their color is deeply affected by processing, due to anthocyanin degradation, which impairs their consumer acceptability.

Anthocyanins are glycosylated polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium, containing two benzoyl rings separated by a heterocyclic ring. Different anthocyanins are characterized by differences in: number and degree of methylation of hydroxyl groups; nature, number and position of sugar moieties attached to the phenolic molecule (aglycone); nature and number of aliphatic or aromatic acids attached to the sugars (Mazza & Miniati, 1993). Anthocyanins are very reactive and degradable; their stability is a function of properties of the product and processing conditions, including chemical structure of the anthocyanins present, light, oxygen, temperature, the presence of enzymes (particularly polyphenol oxidase), proteins, metallic ions, and especially pH (McGhie & Walton, 2007; Patras, Brunton, O'Donnell, & Tiwari, 2010). In aqueous solutions, four different molecular forms of anthocyanins exist in dynamic equilibrium: the red flavylium cation (the most abundant form at pH < 2), the blue quinoidal structure, and the colorless hemiketal and chalcone forms. Although the red flavylium is usually the predominant form in plants, other molecular forms will dominate at neutral pH (McGhie & Walton, 2007).

Acerola anthocyanins are especially susceptible to degradation, which is a problem on storage of juices and purees. The low stability of acerola anthocyanins has been ascribed to the high concentration of ascorbic acid, the degradation occurring by direct condensation of ascorbic acid on C4 of anthocyanins, which results in losses of both components (De Rosso & Mercadante, 2007). Moreover, De Rosso et al. (2008) reported that the aglycones (anthocyanidins) cyanidin and pelargonidin were present in acerolas along with their glycosylated counterparts, which may contribute to the low color stability of acerola products, since aglycones are less stable than glycosylated anthocyanins in weakly acidic medium (He & Giusti, 2010).

Incorporation of organic dyes into inorganic host materials such as

* Corresponding author at: Embrapa Instrumentação, R. 15 de Novembro, 1452, 13560-970 São Carlos, SP, Brazil.
E-mail addresses: edy.brito@embrapa.br (E.S.d. Brito), paulo.riceli@embrapa.br (P.R.V. Ribeiro), men.souza@embrapa.br (M.d.s.M. Souza Filho), henriette.azeredo@embrapa.br (H.M.C. Azeredo).

https://doi.org/10.1016/j.foodchem.2017.11.076
Received 24 July 2017; Received in revised form 17 November 2017; Accepted 20 November 2017
Available online 22 November 2017
0308-8146/

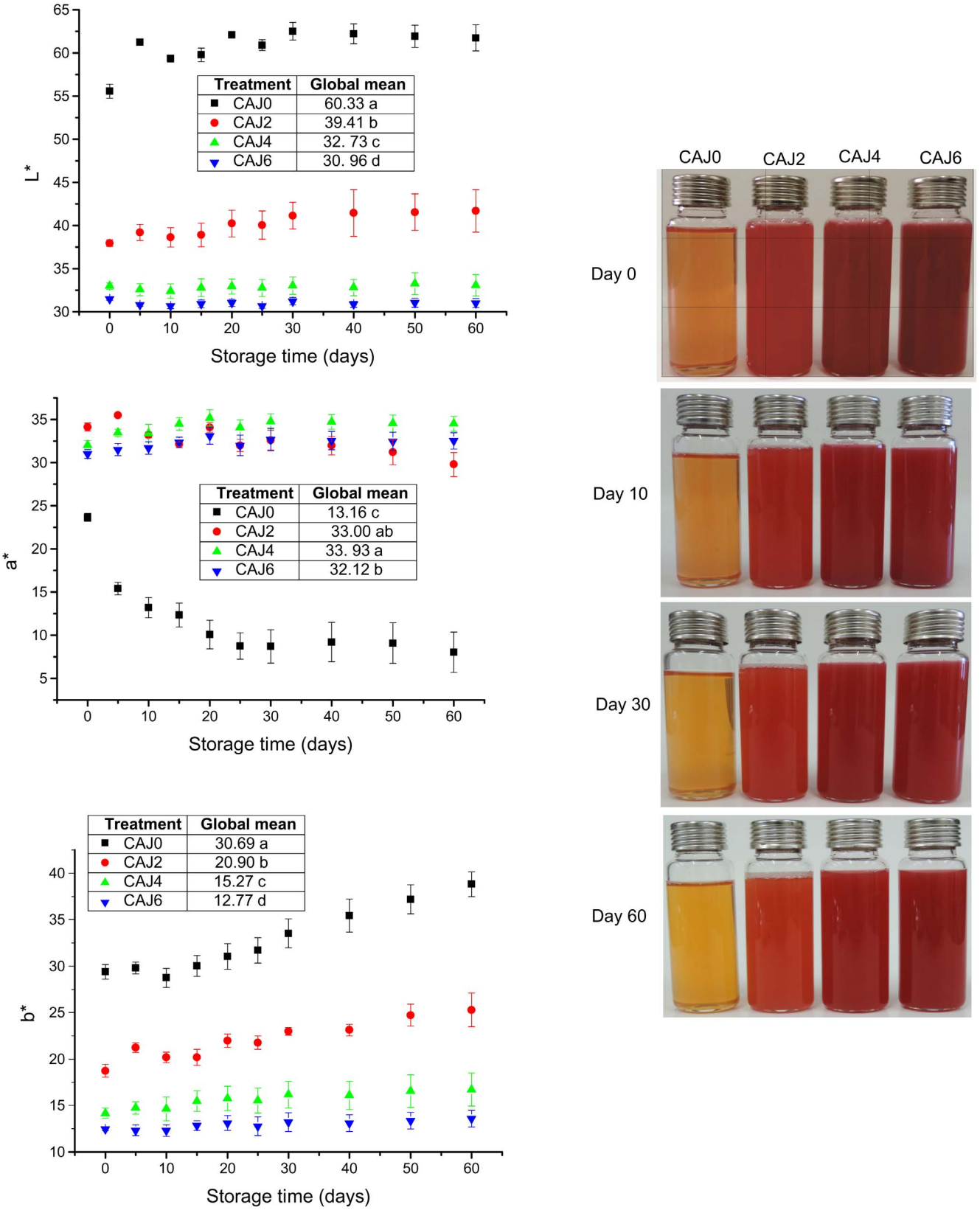
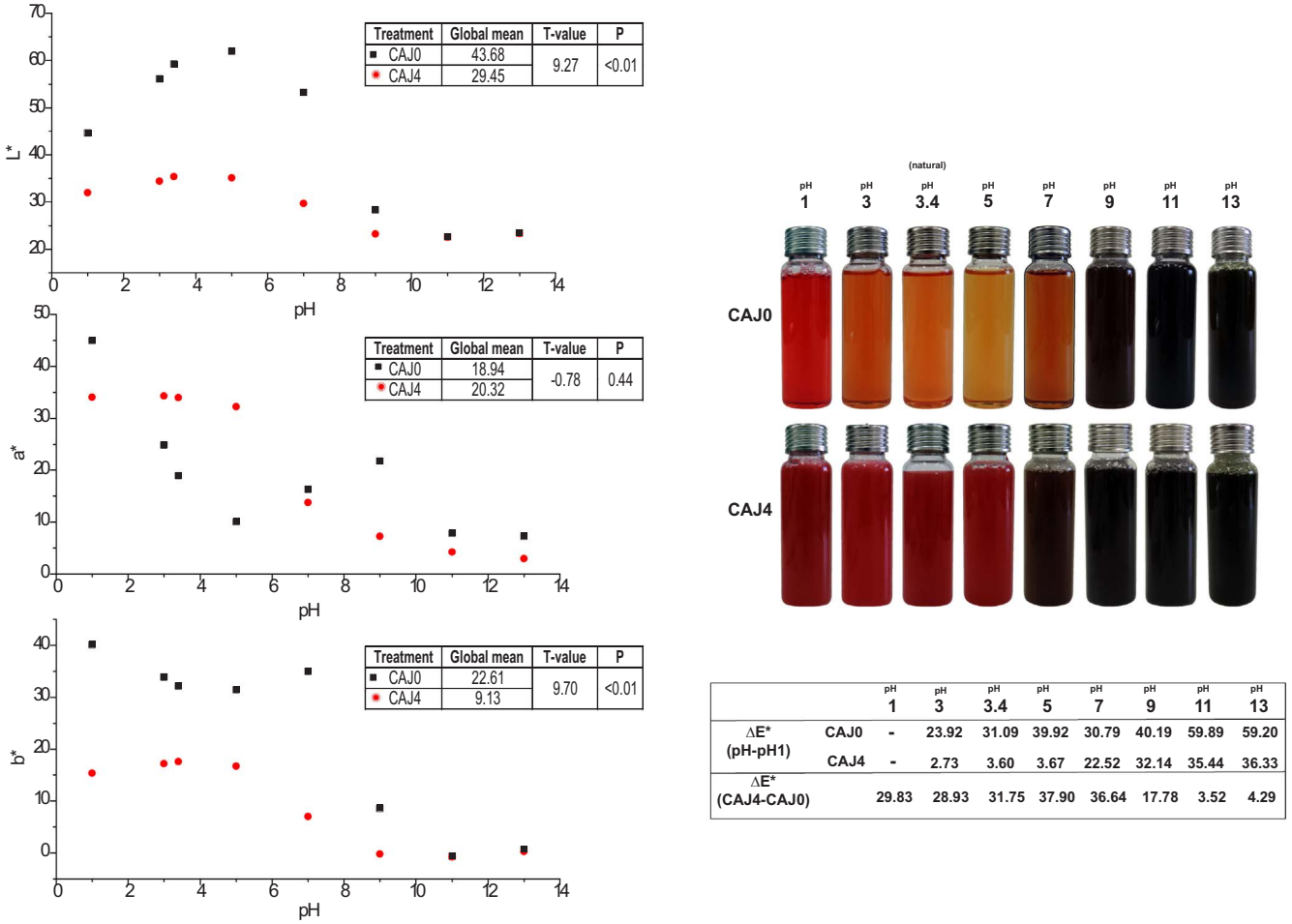


Fig. 1. Color variations of clarified acerola juice with different Mnt concentrations with storage time. Global mean values are averages of 54 measurements in different times, from 0 to 60 days; values followed by the same letter are not different (Tukey, $p < .05$).

clays and zeolites has been reported to enhance color stability (Bujdák, Iyi, & Fujita, 2002; Kohno, Hoshino, Matsushima, Tomita, & Kobayashi, 2007; Kohno et al., 2008; Saito et al., 2005). Kohno et al. (2007) observed that mixing solutions of synthetic flavylium derivatives with Mnt *K*10 resulted in a product with enhanced color stability to high temperatures (up to 80 °C), alkaline pH (~9), and visible light.



		pH 1	pH 3	pH 3.4	pH 5	pH 7	pH 9	pH 11	pH 13
ΔE* (pH-pH1)	CAJ0	-	23.92	31.09	39.92	30.79	40.19	59.89	59.20
	CAJ4	-	2.73	3.60	3.67	22.52	32.14	35.44	36.33
ΔE* (CAJ4-CAJ0)		29.83	28.93	31.75	37.90	36.64	17.78	3.52	4.29

Fig. 2. Color parameters of clarified acerola juice without Mnt (CAJ0) and with 4 wt% Mnt (CAJ4) at different pH values. ΔE^* are the total color differences between CAJ4 and CAJ0 at each pH. Global means of L^*, a^*, and b^* values are averages of all replicates for all pH values.

Table 1
Antioxidant Capacity Trolox Equivalent (TEAC) and ascorbic acid Concentration of clarified acerola juice (CAJ) with different Mnt concentrations.

Treatment	TEAC (μM Trolox/mL juice)	AA (mg/100 g juice)
CAJ0	92.26 ± 2.41	819.39 ± 2.29
CAJ2	52.00 ± 2.57*	798.07 ± 3.09
CAJ4	36.83 ± 2.14*	659.53 ± 48.63*
CAJ6	51.93 ± 1.31*	733.98 ± 11.75

Results were expressed as mean ± standard error of mean. Values in each column followed by an asterisk are significantly different from the control according to Dunnett's test ($p < .05$).

Experiments with X-ray diffraction demonstrated the intercalation of anthocyanins into clay interlayers by cation exchange, and the stabilizing effect was ascribed to the electrostatic host-guest interaction and to steric protection from hydration or isomerization. The same group (Kohno et al., 2009) reported a similar effect when intercalating a natural anthocyanin into Mnt; the absorption peak of the anthocyanin presented a bathochromic shift, from 523 nm to 540 nm, which was attributed to the electrostatic interaction between the clay and the dye.

Mnt clays have attracted attention due to capacity to control the release of drugs (Lee et al., 2005), and also to adsorb dietary toxins (El-Nekeety, El-Kady, Abdel-Wahhab, Hassan, & Abdel-Wahhab, 2017; Shi, Xu, Feng, & Wang, 2006), hydrogen ions in acidosis and metabolic toxins associated with pregnancy (Azhar & Olad, 2014). They have been demonstrated to be non-toxic to rats (Lee et al., 2005). Moreover, they have been considered as clinically safe for several pharmaceutical applications to humans (López-Galindo, Viseras, & Cerezo, 2007). Thus, although those clays are not yet normally used as food additives, there are some evidence that they may be used in a near future.

The objective of this study was to evaluate the effect of Mnt as an additive to stabilize the color of clarified acerola juice.

2. Materials and methods

2.1. Preparation of clarified acerola juice

Frozen acerola puree (Pomar da Polpa, Fortaleza, Brazil) was thawed at 4 °C in a refrigeration chamber, homogenized in an Ultra-Turrax T50 (Ika Labortechnik, Staufen, Germany) at 8000 rpm for 10 min, and centrifuged at 26,400g (Hitachi CR22GIII, Hitachi Koki Co., Japan) for 30 min at 20 °C. The supernatant was vacuum filtered through a 28 μm filter paper, resulting in the clarified acerola juice (hereinafter referred to as CAJ).

2.2. Mnt addition to clarified acerola juice

The Mnt clay (Proenol CN45) was provided by Flow Chemical Ltd. (São Paulo, Brazil). CAJ was added with four different Mnt concentrations (0, 2, 4, and 6 g of Mnt per 100 g on a dry basis of CAJ) was stirred at 660 rpm for 1 h, then homogenized in an Ultra-Turrax T25 (Ika Labortechnik) at 10,000 rpm for 5 min. Each treatment (hereinafter referred to as CAJ0, CAJ2, CAJ4, and CAJ 6, according to the Mnt content) was repeated three times, in volumes of 100 mL each.

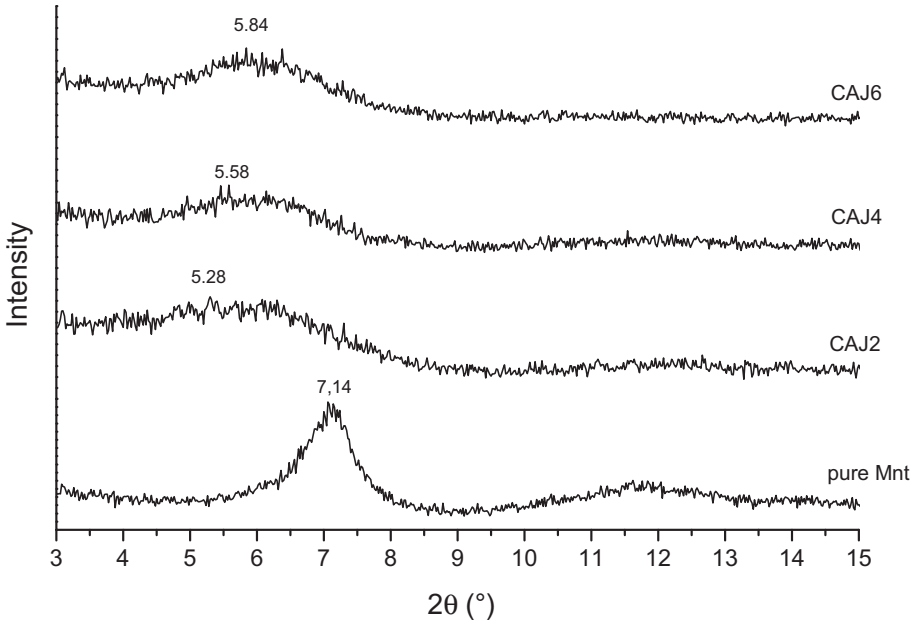


Fig. 3. X-ray diffractograms of Mnt and the precipitates from ultracentrifugation of the juices containing 2, 4, and 6 wt% Mnt (CAJ2-P, CAJ4-P, and CAJ6-P, respectively).

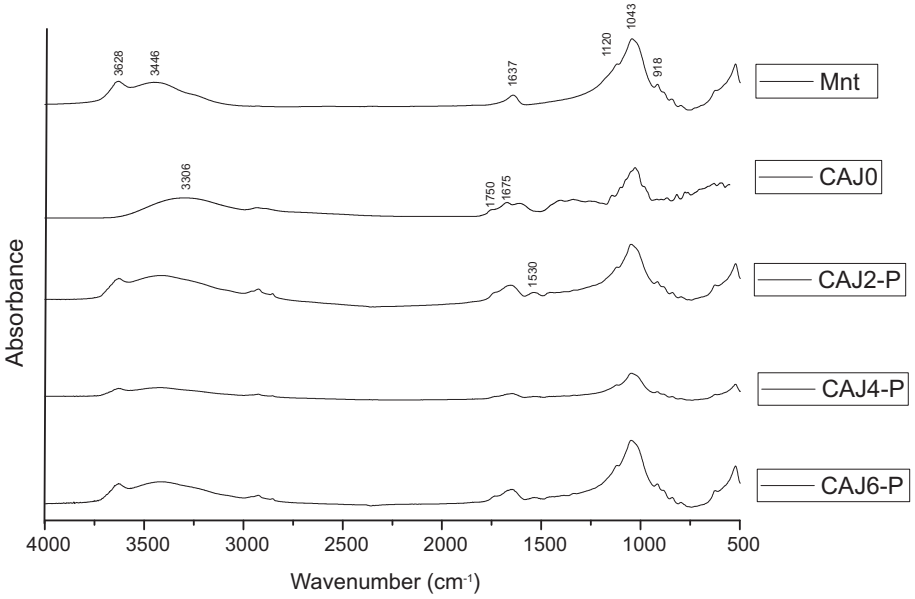


Fig. 4. FTIR spectra of Mnt, clarified acerola juice (CAJ0), and precipitates from centrifuged clarified acerola juices added with different Mnt concentrations (CAJ2 to CAJ6).

2.3. Color stability of clarified acerola juice (CAJ)

2.3.1. Color stability of CAJ during storage

CAJ samples were added with potassium sorbate (0.1%, w/v) under stirring (150 rpm, 15 min) in order to inhibit microbial growth, and stored in a cold chamber at 4 °C. The color of each sample (25 mL sample in 25-mL beakers, over a white background) was measured in triplicate with a Minolta CR-400 Chroma portable colorimeter (Minolta Co., Osaka, Japan). The CIELab color scale was used to measure L^* (lightness, from 0 to 100), a^* (variation from green at negative values to red at positive values), and b^* (variation from blue at negative values to yellow at positive values). The total color difference (ΔE^*) between a CAJ sample containing Mnt (CAJ2, CAJ4 or CAJ 6) and the control (CAJ0) was calculated according to Eq. (1).

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* are the differences in L^*, a^*, and b^* values between the sample and the control.

2.3.2. Color stability of CAJ to pH variation

In order to compare pH-dependent color variations of CAJ samples with or without Mnt, 10 mL samples from both CAJ0 and CAJ4 were transferred to test tubes, and the samples had their pH adjusted to 1, 3, 5, 7, 9, 11, and 13 (by using HCl 1 M or NaOH 1 M). The color parameters (L^*, a^*, and b^*) were measured (in triplicate. Paired t-tests were conducted in order to compare the global means (considering all replicates for all pH values) of the color parameters (L^*, a^*, and b^*) of CAJ0 and CAJ4. Total color differences (ΔE^*) between each CAJ4 sample and the corresponding CAJ0 sample (at each pH) was calculated. Moreover, the ΔE^* between each tested pH and pH 1 (as a reference for anthocyanin stability) were calculated in order to compare CAJ0 and CAJ4 in terms of pH stability.

2.4. Determination of antioxidant activity and ascorbic acid of CAJ

The antioxidant activity of the juices with different Mnt concentrations was determined by ABTS method as described by Rufino

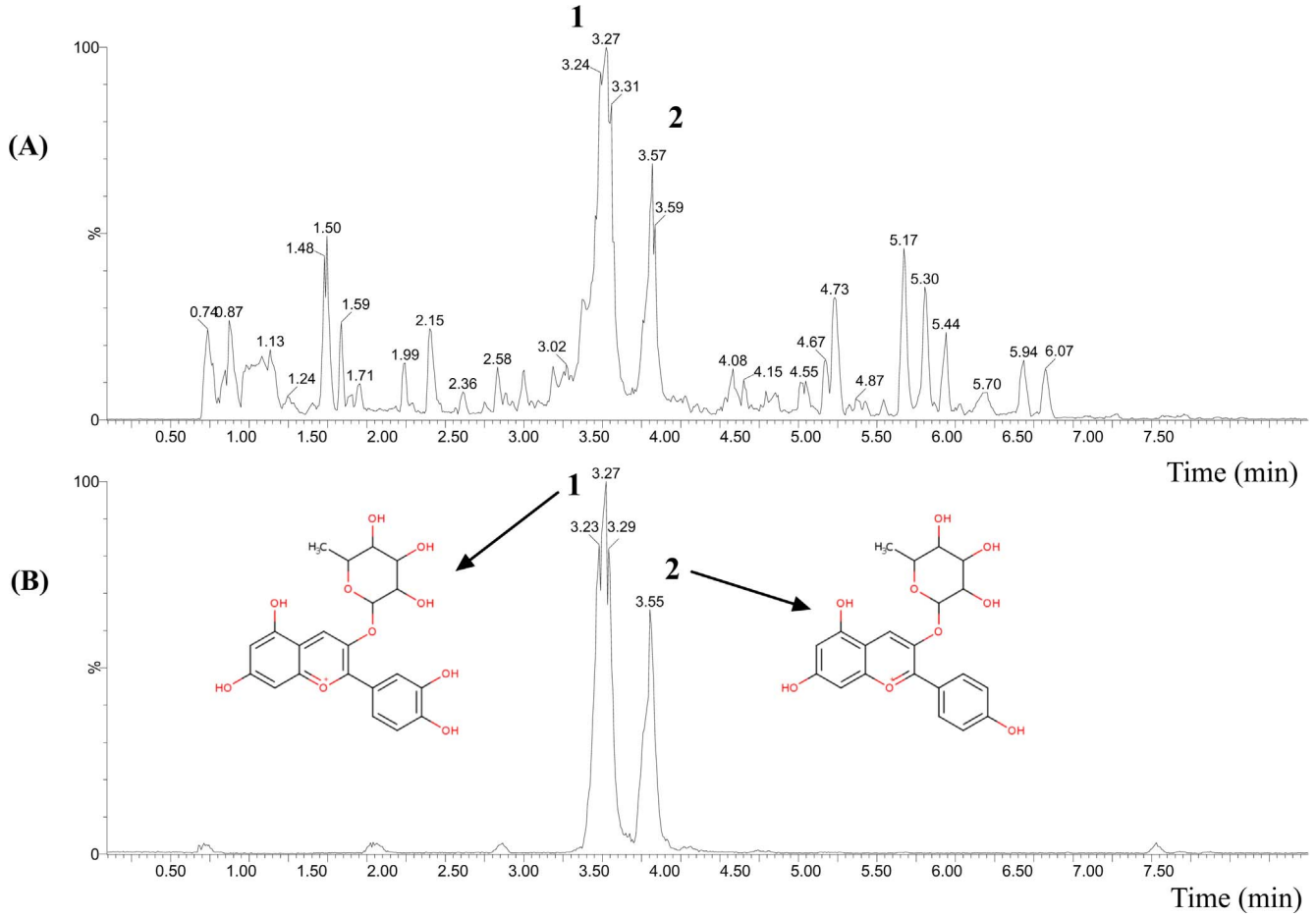


Fig. 5. Chromatogram on the positive mode of CAJ0 (A) and the anthocyanins extracted from CAJ6-P (B); 1) cyanidin-3-*O*-rhamnoside, 2) pelargonidin-3-*O*-rhamnoside.

et al. (2010). The ascorbic acid determination was carried out by HPLC, from 0.5 g samples homogenized with 20 mL of an extracting solution (8% acetic acid, 3% metaphosphoric acid), diluted to 50 mL with distilled water and filtered (0.45 μm). The analyses were performed on a HPLC-DAD equipment consisted of a Shimadzu LC20A HPLC (Shimadzu, Japan) with a SPD-M20A DAD detector (Shimadzu, Japan). A Shim-pack C18 CLC-ODS® Shimadzu (5 μm, 4.6 × 150 mm) reversed phase column was used. The column oven temperature was set at 30 °C. The mobile phase consisted 0.01% sulfuric acid in water at a isocratic flow rate of 1.0 mL/min for 7 min. The DAD was set at 254 nm. Quantification was performed using an external calibration curve of ascorbic acid ranging from 50 to 450 mg/L. Antioxidant activity and ascorbic acid analysis were made in triplicate. The averages of CAJ2, CAJ4, and CAJ6 were compared to that of CAJ0 (control) by Dunnett tests.

2.5. Analysis on supernatants and precipitates from ultracentrifugation

All the CAJ samples containing Mnt (i.e. CAJ2, CAJ4, and CAJ6) were separated into anthocyanin-complexed Mnt phases (precipitates, hereinafter referred to as CAJ2-P, CAJ4-P, and CAJ6-P) and aqueous phases containing compounds not complexed to Mnt (supernatants, hereinafter referred to as CAJ2-S, CAJ4-S, and CAJ6-S) by submitting them to ultracentrifugation in a high-speed centrifuge (Hitachi CR22GIII, Hitachi Koki Co., Japan) at 26,400*g* for 15 min at 4 °C.

2.5.1. Anthocyanin contents of supernatants

The anthocyanin contents of CAJ2-S, CAJ4-S, and CAJ6-S were determined (in triplicate) by the single pH method based on the (absorbance measured at pH 1, as described by Fuleki & Francis, 1968), in order to quantify the anthocyanins which were left uncomplexed, when compared to the total anthocyanin content in the original juice (CAJ0).

2.5.2. X-ray diffraction of precipitates

X-ray diffraction (XRD) is the most common technique to measure the interlayer *d*-spacing of (0 0 1) plane of Mnt. i.e. the distance between its basal layers (Morgan & Gilman, 2003). When a compound intercalates between Mnt layers, the *d*-spacing is expected to increase, so XRD diffractograms of the CAJ precipitates were analyzed in order to indirectly evaluate the capacity of the anthocyanins of intercalating into Mnt layers. The precipitates were left to dry at 25 °C protected from light and grounded to a powder with pestle and mortar.

The X-ray powder diffraction experiments were performed in a Rigaku powder diffractometer (DMAXB) with a Cu Kα (λ = 0.154 nm) radiation tube operated at 40 kV/25 mA. The diffractions were taken in the range of 3–30° (2Θ) in step sizes of 0.02°, and scan speed of 0.25 min^{-1}. The *d*-spacing was calculated from Bragg's law (Eq. (2)).

$$d = \frac{\lambda}{2\sin\theta}, \tag{2}$$

where λ is the radiation wavelength (0.154 nm), and 2θ is the position of the (0 0 1) peak in the XRD pattern.

2.5.3. Fourier Transform Infrared (FTIR) spectra of precipitates

The FTIR spectra of the precipitates, as well as of freeze-dried CAJ0 and pure Mnt, were obtained by using a Shimadzu FTIR-8300 Spectrophotometer (Shimadzu, Kyoto, Japan), in the range of 4000–400 cm^{-1} using KBr pellets.

2.5.4. Liquid chromatography-mass spectrometry

Ten mg of CAJ6-P were suspended in acidified methanol (1 mL methanol + 0.05 mL HCl 6 N), homogenized in a vortex, and centrifuged (HT microcentrifuge CM-610, Hsiang Tai, Taiwan) at 2000*g*, producing a colored supernatant (hereinafter referred to as CAJ6-P-Ant) containing the anthocyanins which had been complexed by Mnt, which was filtered in polytetrafluoroethylene (PTFE) membranes with 0.22 μm pore size (Millipore). Similarly, CAJ0 was filtered and analyzed as well, in order to compare the profile of the anthocyanins complexed by Mnt with the profile of the original clarified juice.

The chromatographic separations were performed on a Waters Acquity UPLC BEH column (150 mm 2.1 mm, 1.7 mm), temperature of 40 °C, mobile phase water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), gradient of 2%–95% B (15 min), flow rate 0.4 mL/min and injection volume of 5 μL. ESI mode was acquired in the range of 110–1180 Da, source temperature fixed at 120 °C, desolvation temperature 350 °C, desolvation gas flow 500 L/h, extraction cone of 0.5 V and voltage capillary of 2.6 kV. ESI^+ mode was acquired in the range of 110–1180 Da, and capillary voltage of 3.2 kV. Leucine enkephalin was used as lock mass. The mode of acquisition was MS^E. The instrument was controlled by Masslynx 4.1 software (Waters Corporation, Waters, Milford, USA).

3. Results and discussion

3.1. Color stability of CAJ during storage

The plain acerola juice suffered noticeable color degradation with time (Fig. 1), mainly in terms of decreasing a^* and increasing b^* values. The sharp decrease in a^* values from the beginning of storage corroborates the behavior reported by De Rosso and Mercadante (2007) for an acerola-based drink. On the other hand, all color parameters were noticeably more stable with storage time when Mnt was present, especially at higher concentrations (at least 4 wt% on a dry basis).

Regarding the global means (taken from 54 measurements from 0 to 60 days for each Mnt content), all juices containing Mnt (CAJ2-CAJ6) presented significant differences in all color parameters when compared to the control (CAJ0). Juices were darker (lower L^* values) and less yellowish (lower b^*) with increasing Mnt concentrations, and redder until 4 wt% Mnt. The total color differences (ΔE^*) between the juices containing Mnt (CAJ2, CAJ4, and CAJ6) and the control (CAJ0) were 30.45, 37.83, and 39.28, respectively.

All color differences between juices with 4 wt% and 6 wt% Mnt were small (although significant), and the reddest juice was the one with 4 wt% Mnt. The reason why the juice with 6 wt% Mnt was less red than the one with 4 wt% Mnt may be related to Mnt competitive self association. So, the juice with 4 wt% Mnt was considered as the most representative of the stabilizing effect of Mnt on acerola juice.

3.2. Color stability of CAJ to pH variation

Fig. 2 presents color differences between CAJ0 and CAJ4 with pH variation. The presence of Mnt made the changes in color parameters less variable as a function of pH, especially for L^* and b^*. Moreover, it was demonstrated from the paired t-tests that the L^* and b^* global means were significantly ($p < .01$) affected by Mnt. Kohno et al. (2009) have already reported the effect of Mnt on color stabilization of anthocyanins at pH values as high as 11, but no previous study has reported color variations of anthocyanin-rich juices added with Mnt along a wide pH range. The total color differences (ΔE^*) and the visual color differences (Fig. 2) between CAJ0 and CAJ4 indicate that the Mnt effects on color were noticeable along the whole acidic to neutral pH range. Moreover, the ΔE^* between all tested pH values and pH1 (most noticeably at the acidic to neutral range) were higher for CAJ4 than for CAJ0, corroborating once more the enhanced color stability of CAJ4 when compared to CAJ0. Those results suggest that Mnt may be useful as a color stabilizer of acerola juice, and perhaps to a variety of other anthocyanin-rich foods.

3.3. Antioxidant activity and ascorbic acid contents

Table 1 presents the antioxidant activity and ascorbic acid determination of CAJ with different Mnt concentrations. The antioxidant activity of the juice was significantly reduced by Mnt addition (at any concentration), which was expected, since the anthocyanins were complexed to Mnt, and therefore not available to contribute to the antioxidant activity of the product. The ascorbic acid content was significantly reduced by 4 wt% Mnt, but surprisingly not by 6 wt% Mnt, which suggests that it might be just an effect from processing (since ascorbic acid is easily degraded) or to Mnt competitive self association, restricting its adsorption capacity.

3.4. Anthocyanin contents of supernatants

The clarified acerola juice without Mnt (CAJ0) presented a total anthocyanin content of 108.72 mg/100 g, while CAJ2-S, CAJ4-S, and CAJ6-S presented 93.76 mg/100 g, 52.20 mg/100 g, and 47.88 mg/100 g respectively, representing decreases in anthocyanin contents of 14%, 52%, and 56% when compared to CAJ0. This result indicates that increasing Mnt concentrations resulted in increasing anthocyanin retention at the Mnt-rich precipitate, although the relationship was not linear. Even when CAJ was added with high Mnt concentrations, the supernatant still retained some anthocyanins, similarly to supernatants from the centrifugation of Mnt-açaí extract (Teixeira-Neto, Izumi, Temperini, Ferreira, & Constantino, 2012).

3.5. X-ray diffractograms of the Mnt-anthocyanin precipitates

Fig. 3 presents the XRD from pure Mnt and the precipitates from ultracentrifugation of CAJ containing different Mnt concentrations. The diffractogram of pure Mnt exhibited a characteristic peak of (0 0 1) plane at $2\theta = 7.14^\circ$, corresponding (according to Bragg's law) to an interlayer space (d_{001}) of 1.24 nm, similar to those reported for Mnt in other studies (Echeverria, Eisenberg, & Mauri, 2014; Joshi, Kevadiya, Patel, Bajaj, & Jasra, 2009; Martucci & Ruseckaite, 2010). For the precipitates CAJ2-P, CAJ4-P, and CAJ6-P, the peaks shifted to lower diffraction angles due to increased interlayer spaces when compared to pure Mnt (1.67, 1.58, and 1.51 nm respectively). Thus, the higher the anthocyanin-rich juice/Mnt ratio, the higher the basal spacing was, confirming anthocyanin intercalation between Mnt layers, corroborating previous results from anthocyanins extracts complexed to smectite clays (Gutiérrez, Ponce, & Alvarez, 2017; Ogawa, Takee, Okabe, & Seki, 2017; Teixeira-Neto et al., 2012).

3.6. FTIR spectra of the precipitates from CAJ ultracentrifugation

The FTIR spectra of Mnt and CAJ precipitates (Fig. 4) shows some bands of the Mnt structure, such as those for –OH stretching of inner hydroxyl groups lying between tetrahedral and octahedral sheets at 3628 cm^{-1} (Madejová, 2003; Xie et al., 2001), in-plane and out-of-plane Si—O stretching at 1043 cm^{-1} and 1120 cm^{-1} respectively (Madejová, 2003; Patel, Somani, Bajaj, & Jasra, 2007; Tyagi, Chudasama, & Jasra, 2006), and bending of aluminum hydroxides around 900 cm^{-1} (Madejová, 2003; Tyagi et al., 2006). The broad band at 3446 cm^{-1} is due to overlapping asymmetric and symmetric H—O—H stretching vibrations of bonded water (Madejová, 2003), and the one at 1637 cm^{-1} is ascribed to H—O—H bending of interlayer water (Madejová, 2003; Xie et al., 2001).

Only a few bands from the acerola juice (CAJ0) are still present in the Mnt-complexed precipitates, such as those for C═O stretching of quinoidal forms of anthocyanins at 1750 cm^{-1} (Luo et al., 2009) and –OH bending of water at 1675 cm^{-1} (Sinelli, Spinardi, Di Egidio,

Mignani, & Casiraghi, 2008). The Mnt-complexed precipitates exhibited a band at 1530 cm^{-1}, which is ascribed to anthocyanin-metal complexes (Buchweitz, Gudi, Carle, Kammerer, & Schulz, 2012). According to those authors (Buchweitz et al., 2012), the band is boosted in presence of metal ions due to chelate formation, with vicinal OH groups in the B-ring of anthocyanins being ligands. Considering that cyanidin-3-rhamnoside have vicinal OH groups in B-ring but not pelargonidin-3-rhamnoside (which has only one OH group in B-ring), this specific band must be due to complexation involving only the former.

3.7. Identification of anthocyanins on clarified acerola juice and the precipitate from CAJ6

The UPLC chromatograms of the anthocyanins extracted from CAJ6-P (Fig. 5B) exhibited two well defined peaks which were part of the chromatogram of CAJ0 (Fig. 5A), indicating the selective adsorption of two compounds by Mnt. Those compounds were identified by LC-MS as cyanidin-3-*O*-rhamnoside ($[M-H]^+$ 433 and a fragment with *m/z* 287) and pelargonidin-3-*O*-rhamnoside ($[M-H]^+$ 417 and a fragment with *m/z* 271), both previously reported on acerola fruits (Brito et al., 2007). Those results, combined with XRD and FTIR analysis, corroborate the capacity of Mnt of selectively adsorbing anthocyanins, which explains the stabilizing effects of Mnt on the color of acerola juice, which have been previously reported for isolating anthocyanins (Kohno et al., 2009; Lima, Martinez-Ortiz, Fregoso, & Mendez-Vivar, 2007; Ogawa et al., 2017) and stabilize films with acerola puree (Azeredo, Miranda, Ribeiro, Rosa, & Nascimento, 2012).

4. Conclusions

Anthocyanins from acerola juice have been demonstrated to intercalate into Mnt interlayers. The intercalation resulted in color changes of acerola clarified juice from a pale red to darker and redder shades, as well as stabilization of its color throughout storage time, even at low acidic pH values. An Mnt concentration of 4 wt% (on a dry basis) was sufficient for intercalation of more than 50% of the anthocyanins and consequent change and stabilization of the red color of the product, although both the antioxidant activity and the ascorbic acid contents have been reduced. Those findings may be useful for color stabilization of a variety of anthocyanin-rich processed foods, as well as for using anthocyanins as stable food colorants.

Acknowledgements

The authors gratefully acknowledge the financial support of the National Collaborative Research Network in Nanotechnology Applied to Agribusiness (AgroNano, Embrapa, Brazil) and the National Council for Scientific and Technological Development (CNPq, Brazil, INCT-Frutos Tropicais, 465335/2014-4). We also thank Flow Chemical Ltd. for providing us with the Mnt clay. Authors H. L. Ribeiro and A.V. Oliveira thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for their MSc scholarships (1708268 and 1376677 respectively). Authors E.S. Brito and H.M.C. Azeredo thank CNPq for their Research Productivity Fellowships (302770/2015-1 and 302381/2016-3 respectively).

Conflict of interest statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

References

Azeredo, H. M. C., Miranda, K. W. E., Ribeiro, H. L., Rosa, M. F., & Nascimento, D. M. (2012). Nanoreinforced alginate-acerola puree coatings on acerola fruits. *Journal of Food Engineering, 113*, 505–510.

Azhar, F. F., & Olad, A. (2014). A study on sustained release formulations for oral delivery of 5-fluorouracil based on alginate-chitosan/montmorillonite nanocomposite systems. *Applied Clay Science, 101*, 288–296.

Brito, E. S., Araújo, M. C. P., Alves, R. E., Carkeet, C., Clevidence, B. A., & Novotny, J. A. (2007). Anthocyanins present in selected tropical fruits: acerola, jambolão, jussara, and guajiru. *Journal of Agricultural and Food Chemistry, 55*, 9389–9394.

Buchweitz, M., Gudi, G., Carle, R., Kammerer, D. R., & Schulz, H. (2012). Systematic investigations of anthocyanin-metal interactions by Raman spectroscopy. *Journal of Raman Spectroscopy, 43*, 2001–2007.

Bujdák, J., Iyi, N., & Fujita, T. (2002). Aggregation and stability of 1,1′-diethyl-4,4′-cyanine dye on the surface of layered silicates with different charge densities. *Colloids and Surfaces A, 207*, 207–214.

Cunha Neto, J., Rabelo, M. C., Bertini, C. H. C. M., Marques, G. V., & Miranda, M. R. A. (2012). Caracterização agronômica e potencial antioxidante de frutos de clones de aceroleira. *Revista Ciência Agronômica, 43*, 713–721.

De Rosso, V. V., Hillebrand, S., Montilla, E. C., Bobbio, F. O., Winterhalter, P., & Mercadante, A. Z. (2008). Determination of anthocyanins from acerola (*Malpighia emarginata* DC.) and açaí (*Euterpe oleracea* Mart.) by HPLC–PDA–MS/MS. *Journal of Food Composition and Analysis, 21*, 291–299.

De Rosso, V. V., & Mercadante, A. Z. (2007). The high ascorbic acid content is the main cause of the low stability of anthocyanin extracts from acerola. *Food Chemistry, 103*, 935–943.

Echeverria, I., Eisenberg, P., & Mauri, A. N. (2014). Nanocomposites films based on soy proteins and montmorillonite processed by casting. *Journal of Membrane Science, 449*, 15–26.

El-Nekeety, A. A., El-Kady, A. A., Abdel-Wahhab, K. G., Hassan, N. S., & Abdel-Wahhab, M. A. (2017). Reduction of individual or combined toxicity of fumonisin B1 and zearalenone via dietary inclusion of organo-modified nano-montmorillonite in rats. *Environmental Science and Pollution Research, 24*, 20770–20783.

Fuleki, T., & Francis, F. J. (1968). Quantitative methods for anthocyanins. Extraction and determination of total anthocyanins in cranberries. *Journal of Food Science, 33*, 72–77.

Gutiérrez, T. J., Ponce, A. G., & Alvarez, V. A. (2017). Nano-clays from natural and modified montmorillonite with and without added blueberry extract for active and intelligent food nanopackaging materials. *Materials Chemistry and Physics, 194*, 283–292.

He, J., & Giusti, M. M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annual Review of Food Science and Technology, 1*, 163–187.

Joshi, G. V., Kevadiya, B. D., Patel, H. A., Bajaj, H. C., & Jasra, R. V. (2009). Montmorillonite as a drug delivery system: Intercalation and in vitro release of timolol maleate. *International Journal of Pharmaceutics, 374*, 53–57.

Kohno, Y., Hoshino, R., Matsushima, R., Tomita, Y., & Kobayashi, K. (2007). Stabilization of flavylium dyes by incorporation in the clay interlayer. *Journal of the Japan Society of Colour Material, 80*, 6–12.

Kohno, Y., Kinoshita, R., Ikoma, S., Yoda, K., Shibata, M., Matsushima, R., ... Kobayashi, K. (2009). Stabilization of natural anthocyanin by intercalation into montmorillonite. *Applied Clay Science, 42*, 519–523.

Kohno, Y., Shibata, Y., Oyaizu, N., Yoda, K., Shibata, M., & Matsushima, R. (2008). Stabilization of flavylium dye by incorporation into the pore of protonated zeolites. *Microporous and Mesoporous Materials, 114*, 373–379.

Lee, Y.-H., Kuo, T.-F., Chen, B.-Y., Feng, Y.-K., Wen, Y.-R., Lin, W.-C., & Lin, F. H. (2005). Toxicity assessment of montmorillonite as a drug carrier for pharmaceutical applications: yeast and rats model. *Biomedical Engineering: Applications, Basis and Communications, 17*, 12–18.

Lima, E., Martinez-Ortiz, M. J., Fregoso, E., & Mendez-Vivar, J. (2007). Capturing natural chromophores on natural and synthetic aluminosilicates. *Studies in Surface Science and Catalysis, 170B*, 2110–2115.

López-Galindo, A., Viseras, C., & Cerezo, P. (2007). Compositional, technical and safety specifications of clays to be used as pharmaceutical and cosmetic products. *Applied Clay Science, 36*, 51–63.

Luo, P., Niu, H., Zheng, G., Bai, X., Zhang, M., & Wang, W. (2009). From salmon pink to blue natural sensitizers for solar cells: *Canna indica* L., *Salvia splendens*, cowberry and *Solanum nigrum* L. *Spectrochimica Acta A, 74*, 936–942.

Madejová, J. (2003). FTIR techniques in clay mineral studies. *Vibrational Spectroscopy, 31*, 1–10.

Martucci, J. F., & Ruseckaite, R. A. (2010). Biodegradable bovine gelatin/Na^+-montmorillonite nanocomposite films. Structure, barrier and dynamic mechanical properties. *Polymer-Plastics Technology and Engineering, 49*, 581–588.

Mazza, G., & Miniati, E. (1993). *Anthocyanins in fruits, vegetables, and grains.* Boca Raton: CRC Press.

McGhie, T. K., & Walton, M. C. (2007). The bioavailability and absorption of anthocyanins: Towards a better understanding. *Molecular Nutrition & Food Research, 51*, 702–713.

Morgan, A. B., & Gilman, J. W. (2003). Characterization of polymer-layered silicate (clay) nanocomposites by transmission electron microscopy and X-ray diffraction: A comparative study. *Journal of Applied Polymer Science, 87*, 1329–1338.

Musser, R. S., Lemos, M. A., Lima, V. L. A. G., Melo, E. A., Lederman, I. E., & Santos, V. F. (2004). Caracteristicas físico-quimicas de acerola do banco ativo de germoplasma em Pernambuco. *Ciência e Tecnologia de Alimentos, 24*, 556–561.

Ogawa, M., Takee, R., Okabe, Y., & Seki, Y. (2017). Bio-geo hybrid pigment; clay-anthocyanin complex which changes color depending on the atmosphere. *Dyes and Pigments, 139*, 561–565.

Patel, H. A., Somani, R. S., Bajaj, H. C., & Jasra, R. V. (2007). Preparation and characterization of phosphonium montmorillonite with enhanced thermal stability. *Applied Clay Science, 35*, 194–200.

Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology, 21*, 3–11.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry, 121*, 996–1002.

Saito, T., Fukui, K., Kodera, Y., Matsushima, A., Nishimura, H., & Inada, Y. (2005). Photostability of biliverdin bound to smectite, clay mineral. *Dyes and Pigments, 65*, 21–24.

SEBRAE (2016). *O cultivo e o mercado da acerola*. Available at < http://www.sebrae.com.br/sites/PortalSebrae/artigos/o-cultivo-e-o-mercado-da-acerola,db7b9e665b182410VgnVCM100000b272010aRCRD > .

Shi, Y. H., Xu, Z. R., Feng, J. L., & Wang, C. Z. (2006). Efficacy of modified montmorillonite nanocomposite to reduce the toxicity of aflatoxin in broiler chicks. *Animal Feed Science and Technology, 129*, 138–148.

Sinelli, N., Spinardi, A., Di Egidio, V., Mignani, I., & Casiraghi, E. (2008). Evaluation of quality and nutraceutical content of blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy. *Postharvest Biology and Technology, 50*, 31–36.

Teixeira-Neto, A. A., Izumi, C. M. S., Temperini, M. L. A., Ferreira, A. M. C., & Constantino, V. R. L. (2012). Hybrid materials based on smectite clays and nutraceutical anthocyanins from the açaí fruit. *European Journal of Inorganic Chemistry, 32*, 5411–5420.

Tyagi, B., Chudasama, C. D., & Jasra, R. V. (2006). Determination of structural modification in acid activated montmorillonite clay by FT-IR spectroscopy. *Spectrochimica Acta A, 64*, 273–278.

Xie, W., Gao, Z., Liu, K., Pan, W. P., Vaia, R., Hunter, D., & Singh, A. (2001). Thermal characterization of organically modified montmorillonite. *Thermochimica Acta, 367–368*, 339–350.