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Research paper

Montmorillonite as a reinforcement and color stabilizer of gelatin films containing acerola juice

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ABSTRACT

Montmorillonite (Mt) is well known as reinforcing agent in films. Moreover, it stabilizes anthocyanin pigments. In this study, Mt has been added as both reinforcement and color stabilizer to gelatin films containing acerola juice. Films were produced with different Mt concentrations (0–6.5% on gelatin). Increasing Mt contents resulted in enhanced tensile strength and modulus, although the elongation has been decreased. Mt also reduced the water vapor permeability in up to 45%. Mt presented two effects on film color: first, it changed its color from yellowish to red; and second, it stabilized film color throughout storage, mainly when added at 3.9%. It has been suggested that metals from Mt (such as Fe^{3+} or Al^{3+}) may have formed complexes to anthocyanins, changing and stabilizing their color.

1. Introduction

Edible films are self-supporting structures produced from edible components, which are usually designed to be used as food wraps, constituting a protective layer additional to the external packaging. Although most proposed edible films are meant not to affect the sensory properties of the wrapped food, films with peculiar sensory properties may be desirable for some specific applications, such as sushi wraps, pizza crust barriers, sachets that melt on cooking, and film snacks (Otoni et al., 2017). In this context, films and coatings added with fruit products (purees or juices) have been particularly well studied (Rojas-Graü et al., 2007; Azeredo et al., 2012a; Otoni et al., 2014; Azeredo et al., 2016), the fruit flavors and colors contributing to the product acceptability.

Acerola (*Malpighia emarginata*) is a very popular tropical fruit, thanks to its high ascorbic acid contents, making it one of the richest sources of vitamin C. The anthocyanins cyanidin-3-rhamnoside and pelargonidin-3-rhamnoside are the main responsible for the red color of acerolas (Brito et al., 2007; De Rosso et al., 2008).

Anthocyanins are very prone to degradation, which may be induced by light, oxygen, temperature, and especially pH changes (McGhie and Walton, 2007; Patras et al., 2010). In aqueous solutions, anthocyanins co-exist as four species in equilibrium – flavylium cation (red, the main form at pH < 2), quinonoidal base (blue), carbinol or pseudobase (colorless), and chalcone C (colorless) (Pascual-Teresa and Sanchez-Ballesta, 2008). Some anthocyanins are relatively stable, such as the acylated ones (Guldiken et al., 2017), since acylation promotes an increase in the relative proportion of the flavylium cation (Pascual-Teresa and Sanchez-Ballesta, 2008). On the other hand, some other pigments are especially degradable, including acerola anthocyanins, making the red color of the fruit to dramatically change on processing and storage (Mercali et al., 2013; Mercali et al., 2014), acquiring yellowish or brownish colors. The high degradability of acerola anthocynins has been ascribed to direct condensation of ascorbic acid on C4 of anthocyanins (De Rosso and Mercadante, 2007) and/or to the presence of aglycones (anthocyanidins) (De Rosso et al., 2008), which are less stable than the corresponding glycosylated anthocyanins (He and Giusti, 2010).

Some inorganic compounds have been reported to change color and enhance color stability of anthocyanins. Flavylium derivatives and an anthocyanin were demonstrated to present enhanced color stability when in solution with clay minerals like montmorillonite (Mt) (Kohno et al., 2007; Kohno et al., 2009), which was ascribed to electrostatic host-guest interaction and steric protection resulting from the intercalation of anthocyanins into Mt interlayers by cation exchange. Unmodified Mt has been reported as non-toxic to human intestinal cell line Caco-2 (Maisanaba et al., 2014), and has been considered as safe to be added to human diets to decrease the bioavailability of aflatoxins (Wang et al., 2007).

In a previous study from this group (Ribeiro et al., 2018), Mt was

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demonstrated to intercalate anthocyanins from clarified acerola juice, which resulted in a dramatic color change (from yellowish to bright red) and color stabilization of the juice. The objective of the present study was to evaluate the effectiveness of Mt to act as both color stabilizer and reinforcing phase to gelatin films containing 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 26,400*g* (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 clarified acerola juice.

2.2. Preparation of gelatin films

Each film was prepared from 25 g bovine gelatin powder (Bloom value 226, PB Leiner, Acorizal, Brazil), 250 mL clarified acerola juice (with 6.5 wt% solids), 12.5 g glucose syrup with 80% glucose (Du Porto, Porto Feliz, Brazil) as a film plasticizer and sweetener, and Mt (Proenol CN45, Flow Chemical, São Paulo, Brazil, an aluminum silicate containing the following chemical composition, as informed by the manufacturer: SiO_2, 66.0%; Fe_2O_3, 3.0%; CaO, 1.0%; TiO_2, 0.8%; Al_2O_3, 19.5%; MgO, 5.0%; Na_2O, 3.0%; K_2O, 0.1%). The following Mt contents were defined for the films: 0, 2, 4, 6, 8, and 10 wt% when based on dry acerola juice, which turned out to be 0, 1.3, 2.6, 3.9, 5.2, and 6.5 wt% respectively, when based on the gelatin matrix.

Mt was firstly hydrated in clarified acerola juice under stirring (660 rpm) for 30 min, then sonicated in an ultrasonic cell disruptor (DES500, Unique Group, Indaiatuba, Brazil) at a frequency of 20 kHz for two 5 min cycles at 500 W with a 5 min interval in between. The gelatin was then added, and the mixture was stirred (660 rpm) for 30 min at 25 °C, then for more 30 min at 50 °C. The glucose syrup was then added, and the dispersion was stirred (660 rpm) for 30 min, homogeneized in an Ultra-Turrax T-25 (Ika, Staufen, Germany) at 10000 rpm for 15 min, sonicated (20 kHz, 500 W, 5 min), and degassed with a vacuum pump (DVP RC.8D, Vacuum Technology, Italy) to remove bubbles. The dispersion was then cast on Mylar® films fixed on 30 × 30 cm glass plates, leveled with a draw-down bar to a final dry thickness of 0.08 mm, and left to dry at 24 °C for 24 h. Dried samples were cut and detached from the Mylar surface.

2.3. Determinations on gelatin films

2.3.1. Tensile tests

At least ten 125 × 12.5 mm film strips were conditioned for 48 h under controlled humidity (50 ± 5 °C) and temperature (23 ± 1 °C) and had their thicknesses measured with an Akrom KR1250 coating thickness tester (Akrom, São Leopoldo, RS, Brazil) to the nearest 1 μm at 7–8 random locations. The strips had their tensile properties measured according to the D882–12 method (ASTM, 2012), using an Emic DL-3000 Universal Testing Machine (Emic, São José dos Pinhais, Brazil) with a load cell of 100 N, initial grip separation of 100 mm, and crosshead speed of 12.5 mm.min^{-1}.

2.3.2. Water vapor permeability (WVP)

Six circular (30 mm in diameter) film samples, previously conditioned (48 h, 50 ± 5 °C, 23 ± 1 °C) and with their thicknesses measured at 7–8 random locations, were submitted to water vapor permeability (WVP) determination according to the E96–05 method (ASTM, 2016). The film samples were sealed as patches onto acrylic permeation cells (24 mm in diameter and 10 mm in height) containing 2 mL distilled water. The test was carried out at 24 °C, using silica gel in the desiccator (outside the permeation cells). Eight measurements were taken within 24 h.

2.3.3. Opacity

Film opacity determination (in triplicate) was based on a method described by Irissin-Mangata et al. (2001). Films were cut into rectangular (10 × 50 mm) strips and placed on the internal side of a UV–visible spectrophotometer cell (perpendicularly to the light beam), and the absorbance spectrum (400–800 nm) of film samples was recorded on a Shimadzu UV-2450 spectrometer (Shimadzu, Japan) equipped with an integrating sphere (ISR-2200, Shimadzu, Japan). Flm opacity was defined as the area under the recorded curve (estimated by the linear trapezoidal rule) and expressed as absorbance units × nanometers (wavelength)/millimeters (film thickness) (A.nm.mm^{-1}).

2.3.4. Color parameters and stability

Film forming dispersions for color analyses were prepared as described previously (item 2.2), except that 0.1% (*w/v*) potassium sorbate was added in order to avoid microbial growth[1] (which might change the material color). 25 mL of each dispersion were cast on a 90-mm petri dish. The following color parameters of the films were measured: lightness (L*, ranging from 0 to 100, representing black and white, respectively), red–green chromaticity (a*, negative values indicating green and positive values indicating magenta), and yellow–blue chromaticity (b*, negative values indicating blue and positive values indicating yellow). Color measurements were carried out with a Konica–Minolta CR-400 colorimeter (Minolta, Colombes, France) standardized with a white reference plate (L* = 95.62, a* = −0.22 and b* = 2.45). Measurements were taken (as the average of five points of each sample) daily for the first 8 days of storage at 24 °C, and another measurement was taken at 18 days of storage. Total color differences (ΔE*) were calculated according to Eq. 1, to assess the color variation throughout storage time.

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

where ΔL*, Δa*, and Δb* are the differences in L*, a*, and b* values after 17 days of storage when compared to the beginning of storage (day 0).

2.3.5. X-ray diffraction

X-ray diffraction (XRD) was used to assess the interlayer *d*-spacing of (001) plane of Mt, i.e. the distance between its basal layers (Morgan and Gilman, 2003), in order to indirectly evaluate the capacity of Mt of intercalating film components.

The X-ray powder diffraction experiments were performed for films containing 0, 3.9, and 6.5 wt% Mt (on a gelatin basis) in a Rigaku diffractometer (DMAXB) with a Cu Kα (λ = 0.154 nm) radiation tube operated at 40 kV/25 mA. The diffractions were taken in the 3–35° (2θ) range 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)).

$$d = \frac{\lambda}{2sin\theta} \quad (2)$$

where λ is the radiation wavelength (0.154 nm), and 2θ is the position of the (001) peak in the XRD pattern.

2.3.6. Fourier Transform Infrared (FTIR) spectra

The FTIR spectra of films and pure Mt were recorded with a Perkin

[1] Microbial growth was a concern for this specific analysis, since a 0.4 mm-thick layer was to be obtained (much higher than the 0.08 mm for films) in order to minimize color interferences from the bottom (reference plate), and it took much longer (72 h) for this layer to dry when compared to the regular films (24 h).