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Nanoreinforced alginate–acerola puree coatings on acerola fruits

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ABSTRACT

Combinations of fruit purees with polysaccharides have been explored to produce edible films and coatings. In this study, the combination between acerola puree and alginate was reinforced with cellulose whiskers (CW) or montmorillonite (MMT) to form nanocomposite edible films (casted on glass plates) and edible coatings (applied on acerola fruit surfaces). Three film/coating dispersions were formulated, based on unfilled alginate–acerola puree (AA), CW-reinforced alginate–acerola puree (CWAA), and MMT-reinforced alginate–acerola puree (MMTAA). Both nanofillers (CW and MMT) reduced water vapor permeability (WVP) of films. When applied to fresh acerolas, the coatings decreased fruit weight loss, decay incidence, and ripening rates. Ascorbic acid retention by the fruits were favored by the coatings, especially the nanocomposite ones. The MMTAA coating was the most effective in reducing weight loss of acerolas. Moreover, it was the coating which best maintained its red color and the visual acceptance of coated acerolas.

1. Introduction

Difficulties in disposing of the huge waste volumes generated by non-biodegradable food packaging have motivated the study of biopolymers as materials to be used as edible coatings in food packaging. Edible coatings are not supposed to completely replace conventional food packaging, but they can help the packaging in the function of food preservation.

Important functionalities of edible coatings for fresh fruits include: moderate oxygen permeability, modifying the internal atmosphere of fruits, delaying senescence (Rojas-Graü et al., 2009); low water vapor permeability, preventing desiccation and maintaining fruit firmness (Ayranci and Tunc, 2004; Del-Valle et al., 2005; Han et al., 2004); mechanical protection, reducing injury effects; and sensory appeal.

Film forming properties of alginate are related to its ability to form strong gels or insoluble polymers in the presence of multivalent metal cations like Ca^{2+} (Mancini and McHugh, 2000; Rhim, 2004). The gelling mechanism involves interactions between Ca^{2+} and carboxylic groups of alginate, forming a three-dimensional cross-linked network (Oms-Oliu et al., 2008). Alginate coatings, as other polysaccharide-based coatings, are expected to present low oxygen permeabilities due to their ordered hydrogen-bonded network structure. On the other hand, polysaccharides coatings are not good moisture barriers because of their hydrophilic nature (Nisperos-Carriedo, 1994; Yang and Paulson, 2000). Indeed, the main effect of polysaccharide-based coatings on shelf-life of fruits is that of reducing respiration rates due to selective permeabilities to O_2 and CO_2 (Nisperos-Carriedo, 1994; Maqbool et al., 2011).

Acerola is a red fruit whose demand has increased in the last decades, thanks to its high ascorbic acid (Johnson, 2003). However, because of the short postharvest shelf life of the fresh fruit, it is mainly commercialized as puree. Some studies have described the production of edible films (Azeredo et al., 2009; Rojas-Graü et al., 2006, 2007; Senesi and McHugh, 2002) and coatings (Sothornvit and Rodsamran, 2008) from fruit purees, combined or not with polysaccharides. The presence of polysaccharides such as pectin and starch in fruit purees is responsible for their film forming ability (Kaya and Maskan, 2003). The development of films and coatings from fruit purees is an interesting way of combining the mechanical and barrier properties provided by those polysaccharides with the sensory and nutritional properties of the fruit. The high polarity of fruit puree based coatings make them relatively good barriers to O_2, but poor barriers to water vapor.

Mechanical and water vapor barrier properties of biopolymer films can be improved by adding nanoreinforcements, such as cellulose whiskers (Azizi Samir et al., 2005; Saxena and Ragauskas, 2009) or layered silicates (Cyras et al., 2008; Kampeerapappun et al., 2007). In some previous studies, cellulose nanoreinforcements have demonstrated to improve water vapor barrier and

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tensile properties of mango (Azeredo et al., 2009) and acerola (Azeredo et al., 2012) puree based films.

The objectives of this study were: (a) to compare water vapor barrier of two nanocomposite films based on alginate–acerola puree (AA) matrix with those of an unfilled AA film; (b) to evaluate the ability of unfilled and nanocomposite coatings to enhance acceptance and stability of fresh acerolas.

2. Materials and methods

2.1. Film/coating materials and formulations

Two nanofillers were used to formulate nanocomposite coatings, namely: (a) the montmorillonite-type layered silicate Proenol CN 45 (provided by Flow Chemical Ltd., São Paulo, SP, Brazil), containing the following chemical composition (w/w, as informed by the provider): 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%; and (b) cellulose whiskers (average dimensions, 5.5 nm in diameter and 194 nm in length, as determined previously by our group, as described in Azeredo et al., 2012) extracted from coconut husk fibers by a 120-min hydrolysis preceded by one-stage bleaching (Rosa et al., 2010). In addition to the two nanocomposite coatings (cellulose whiskers reinforced alginate–acerola puree coating, or simply CWAA, and montmorillonite reinforced alginate–acerola puree coating, or MMTAA), an unfilled alginate–acerola puree coating (AA) was also formulated.

For the unfilled AA coating formulation, 1.6 g sodium alginate (Grinsted® FD175, provided by Danisco Brasil Ltd.), and 4 g of corn syrup (Karo, Unilever, São Paulo, SP, Brazil) were added to 100 g of acerola puree (AliPolpa, Aquiraz, CE, Brazil, with 6.4% total solids) and 50 mL of distilled water. Corn syrup was used as plasticizer and sweetener. The formulation was based on preliminary tests. The CWAA and MMTAA dispersions were formulated by the same procedure, including the addition of 10% (w/w, based on solid matter content of films, including alginate, acerola puree, and corn syrup solids) of cellulose whiskers (CW) or montmorillonite (MMT), respectively. The amount of added distilled water was adjusted in each case, so as to obtain film forming dispersions with the same solid contents (i.e., about 8.4%).

The plasticizer (corn syrup) was added in order to break polymer–polymer interactions (such as hydrogen bonds and van der Waals forces) and to form secondary bonds to polymer chains, causing the distance between adjacent chains to increase, thus reducing film rigidity and brittleness (Sothornvit and Krochta, 2005). Corn syrup is basically constituted by simple carbohydrates, being thus well suited to a hydrophilic matrix such as the alginate–acerola puree combination used in this study.

For all film formulations, the mixtures were homogenized in a magnetic stirrer (Fisatom 752A, Aaker Solutions Ltd., Porto Alegre, Brazil) for 60 min at 200 rpm, 50 °C, and then in a cell disruptor (DES500, Unique Group, Indaiatuba, SP, Brazil) for 18 min at 90 W. The mixture was vacuum degassed (at 25 °C), and used to form both films and coatings. Films were formed only to have their water vapor permeability (WVP) analyzed, in order to correlate this measurement with the responses obtained for the coated acerolas.

2.2. Film formation

For film formation, dispersions were cast on 30 × 30 cm glass plates and leveled with a draw-down bar to a thickness of 1.2 mm. After 1 h, the plates with the film dispersions were immersed for 15 s in a 2% (w/v) calcium chloride ($CaCl_2$) solution to crosslink sodium alginate in order to obtain films with better water resistance and barrier. Higher immersion times would possibly produce even better water resistance and decreased water vapor permeability, but previous tests revealed that the resulting films would be noticeably salty, and could impair the acceptability of coated acerolas. The films were placed on a lab bench (24 ± 1 °C, RH 76 ± 2%) for 24 h to dry. Then, samples were cut and detached from the surface.

2.3. Water vapor permeability tests

Prior to WVP determination, the detached, free-standing films were conditioned for 24 h at 25 °C in desiccators containing $Mg(NO_3)_2$ saturated solution (53% RH) (Tunç and Duman, 2007). The WVP determination, with eight replicates, was based on the method E96-80 (ASTM, 1989) at 24 °C and 85% RH, using silica gel as the desiccant material, and at least seven measurements within a 24-h period.

2.4. Coating formation

Fresh acerolas (21.2 mm in diameter, 23.2 mm in height, 4.24 g in mass)[1] were washed, sanitized by immersion in a sodium hypochlorite solution (200 mg L^{-1}), hung from their stalks, and sequentially immersed in the coating formulation (AA, CWAA or MMTAA) for 2 min, and in a $CaCl_2$ solution (2 g/100 mL) for 15 s. After treatment, fruits were placed on a bench at 25 °C, RH 76% for 24 h to allow the coating to dry. They were then weighted, packed in polyethylene terephthalate (PET) trays, and stored in a refrigerator at 6 °C. A control group of uncoated acerolas (U), also previously washed and sanitized, was stored under the same conditions. Acerolas from the four groups (U, AA-coated, CWAA-coated, and MMTAA-coated) were subjected to the determinations described in 2.5–2.9. Acceptance was determined only at day 0, and the other determinations were carried out at day 0 and after 7 days of storage. Except for the sensory analysis, all the tests were conducted in quintuplicate.

2.5. Visual acceptance

Acerolas were tested for visual acceptance, according to Meilgaard et al. (2007). Fifty non-trained panelists took part in the test, in which the visual acceptance was evaluated by using 9-Point Hedonic Score System, with ratings ranging from 1 (“disliked extremely”) to 9 (”liked extremely”). Since a coating is an integral part of the food and consumed as such, and considering that the nanoreinforcements are not yet permitted as food additives or ingredients, flavor and texture acceptance tests were not carried out. Specific toxicity tests are still required in order to establish the safety of such nanoreinforcements as edible materials.

2.6. Weight loss and decay incidence

Weight loss was calculated as percentage on a fresh weight basis. The decay incidence of acerolas was expressed as percentage of visibly decayed fruits (El-Anany et al., 2009).

2.7. Total soluble solids and total titratable acidity

Acerolas were manually ground, and the pulp was used to determine total soluble solids (TSS) and total titratable acidity (TTA). TSS content was measured by using a digital refractometer (PR-101, Atago, Japan). TTA was determined according to AOAC (2005).

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[1] Average measurements from 20 acerolas.

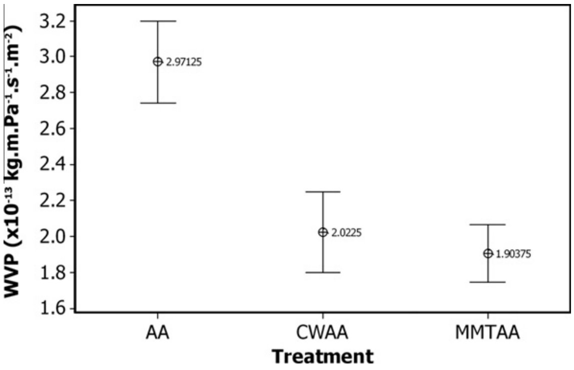

Fig. 1. Water vapor permeability of films formed from the unfilled (AA) and both nanoreinforced (CWAA and MMTAA) coating formulations.

2.8. Ascorbic acid losses

Ascorbic acid contents were measured by redox titration with 2,6-dichlorophenol–indophenol (AOAC method 967.12, AOAC, 2005). For each storage time, total solids content of the acerolas was determined by the oven-drying method at 105 °C until a constant weight was reached, and ascorbic acid losses were calculated on a dry basis.

2.9. Color

Color parameters were measured not only on coated and uncoated acerolas, but also on the acerola puree used for the experiment. Color was determined using the Hunter Lab System, by a Chroma Meter CR-300 (Konica Minolta Sensing Inc., Osaka, Japan). Values were recorded as lightness (L^*, ranging from 0 to 100, corresponding to black to white, respectively), chroma (C^*, representing color intensity or saturation), and hue angle (H^*, representing red–purple at 0°, yellow at 90°, bluish-green at 180°, and blue at 270°, according to Jha, 2010). Measurements were taken from two points along each of 10 acerolas, and in quintuplicate on the puree. Values for C^* and H^* were calculated from the measured parameters a^* (red) and b^* (yellow), using Eqs. (1) and (2).

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{1}$$

$$H^* = \arctan\frac{b^*}{a^*} \tag{2}$$

2.10. Statistical analyses

The data were subjected to analysis of variance (ANOVA) using the software Minitab® 15 (Minitab Inc., State College, PA, USA) in order to establish differences among treatments. For the attributes whose F values were significant, Tukey's multiple range tests were used to compare means at the 95% confidence level.

3. Results and discussion

3.1. Water vapor permeability

The films presented a final average thickness of 0.10 mm. Fig. 1 presents the results for the determinations of water vapor permeability (WVP) of films, indicating that both the nanocomposite films presented improved water vapor barrier when compared to that of the unfilled AA film. This finding corroborates other studies which have reported enhanced water vapor barrier of polymers by cellulose nanoreinforcements (Azeredo et al., 2010; Paralikar et al., 2008; Svagan et al., 2009; Bilbao-Sainz et al., 2011) and MMT clays (Müller et al., 2011; Park et al., 2002; Strawhecker and Manias, 2000). Indeed, the presence of clays such as MMT in polymer formulations increases the tortuosity of the diffusive path for a penetrant molecule, enhancing barrier properties (Cabedo et al., 2004; Mirzadeh and Kokabi, 2007). Similarly, the presence of nanocrystalline reinforcements such as cellulose whiskers is thought to increase the tortuosity in the materials leading to slower diffusion processes and, hence, to lower permeability (Sanchez-Garcia et al., 2008). Moreover, cellulose–water interactions result in rearrangements of water molecules within the matrix, decreasing the plasticization effect of water (Roohani et al., 2008; Song and Zheng, 2009), and consequently favoring the barrier properties of the material. The barrier properties are enhanced if the fillers have high aspect ratios and are well dispersed in the matrix (Lagaron et al., 2004). In the present study, the hydrophilic nature of both cellulose whiskers and montmorillonite favored their interactions with the alginate–acerola puree matrix and the plasticizer.

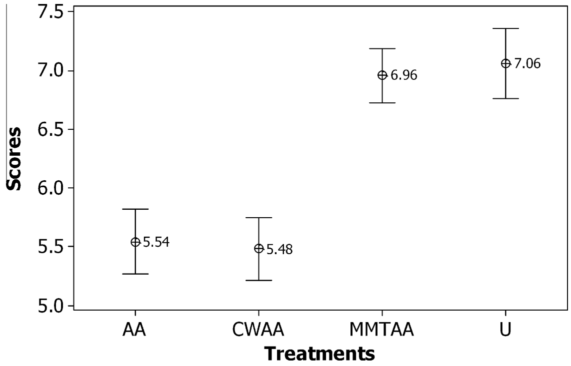

Fig. 2. Visual acceptance of acerolas submitted to different coatings treatments.

3.2. Visual acceptance and color

AA- and CWAA-coated acerolas were less accepted than uncoated fruits, whereas MMTAA-coated acerolas was as well accepted as those (Fig. 2). The puree used for the coatings exhibited color properties ($L^* = 27.14$; $H^* = 30.04$; and $C^* = 36.83$) quite similar to those of uncoated acerolas (Table 1). However, AA- and CWAA-coated acerolas exhibited higher L^* and H^* values and lower C^* values than those of uncoated fruits (Table 1). On the other hand, MMTAA-coated acerolas presented color properties very similar to those of fresh acerolas.

The differences in acceptance of the acerolas were probably explained by the different colors of the coatings. Although the puree used for the coatings exhibited a bright red color, acerola purees (and consequently the puree-based coatings) are highly susceptible to color fading, because of anthocyanin degradation during processing and storage (Bakowska et al., 2003), which results in color changes (Steyn et al., 2004). Indeed, the brownish pale-red color of AA- and CWAA-coated acerolas indicates loss of saturation and partial conversion of flavylium cation of the anthocyanins into colorless forms (De Rosso and Mercadante, 2007; Rodríguez-Saona et al., 1999), which explains why they were less accepted. On the other hand, MMTAA coating maintained the acerola puree color properties, and thus its visual acceptance. This observation corroborates other studies which have described an anthocyanin stabilizing effect of MMT. Kohno et al. (2007) observed the stabilization of a synthetic flavylium cation by intercalation into MMT, which was achieved by simply mixing a flavylium aqueous solution with MMT. The dye was stabilized by the electrostatic field between clay platelets, and the steric effect inhibited hydration or isomeri-

Table 1
Influence of the different coatings on color attributes of acerola surfaces.

Determination	Storage time (days)	Treatments			
		U	AA	CWAA	MMTAA
L^* values	0	26.78c	38.95a	35.54b	27.56c
	7	31.53c	40.90a	36.78b	28.47d
H^* values	0	32.89b	46.79a	46.43a	29.93c
	7	35.22b	48.99a	48.31a	32.36c
C^* values	0	36.39a	29.00b	29.20b	35.81a
	7	33.84a	27.20b	26.97b	33.61a

U: uncoated acerolas; AA: acerolas with unfilled alginate–acerola puree; CWAA: acerolas with cellulose whiskers reinforced alginate–acerola puree coatings; MMTAA: acerolas with montmorillonite reinforced alginate–acerola puree coatings. Values in the same row followed by the same letter are not significantly different ($p < 0.05$).

Table 2
Influence of the different coatings on acerola stability after a 7-day storage.

Determination	Treatments			
	U	AA	CWAA	MMTAA
Weight loss (%)	11.13a	7.19b	5.17c	4.36d
Decay (%)	43.3a	20.0b	23.3b	16.7b
Total soluble solids (%)	8.70a	7.33b	7.17b	7.13b
Total titratable acidity ([H$^+$], in mol L^{-1})	0.51b	0.61a	0.60a	0.61a
Ascorbic acid loss (%)	22.69a	8.83b	6.73c	6.45c

U: uncoated acerolas; AA: acerolas with unfilled alginate–acerola puree; CWAA: acerolas with cellulose whiskers reinforced alginate–acerola puree coatings; MMTAA: acerolas with montmorillonite reinforced alginate–acerola puree coatings. Values in the same row followed by the same letter are not significantly different ($p < 0.05$).

zation reactions. MMT has been confirmed by Kohno et al. (2009) and Lima et al. (2007) to adsorb and stabilize anthocyanins. According to Kohno et al. (2009), the intercalated anthocyanin exhibits enhanced stability against visible light and maintains its original color at pH as high as 11.

3.3. Weight loss

Table 2 presents stability parameters of coated and uncoated acerolas after 7 days of storage. Coated acerolas presented lower weight loss than uncoated fruits. Nanocomposite coatings were more effective than unfilled coating in reducing the weight loss of acerolas; when comparing nanocomposite coatings, MMTAA was more effective than CWAA.

The reduced fruit weight loss from incorporation of coatings corroborates previous results associated to polysaccharide coatings (Chien et al., 2007; Han et al., 2004; Hernández-Muñoz et al., 2008; Ribeiro et al., 2007), including a fruit puree coating (Sothornvit and Rodsamran, 2008). The further reduction of fruit weight loss by the nanofillers is in agreement with the fact that both nanofillers improved the water vapor barrier of the films. On the other hand, although the WVP decreasing effects of both nanofillers on the films were not significantly different from each other (Fig. 1), the higher weight loss reducing effect of MMTAA coating when compared to CWAA coating may probably result from a difference in O_2 permeability of the nanocomposite coatings. Indeed, MMT has been reported to be more effective in reducing O_2 permeability of polymers than cellulose nanoreinforcements (Petersson and Oksman, 2006), thus MMTAA-coated acerolas may have had the lowest respiration rates. Since fruit weight loss is associated not only with moisture evaporation through the skin but also with respiration (Hernández-Muñoz et al., 2008), the difference in O_2 permeability of the nanocomposite coatings is most probably the explanation for the lowest weight loss in MMTAA-coated acerolas.

3.4. Decay

The decay percentage of the coated acerolas was lower than that of the uncoated ones (Table 2). No significant differences were observed among the different groups of coated acerolas.

Previous studies have demonstrated that other polysaccharide coatings also reduced decay incidence in fruit (Baldwin et al., 1999; Hernández-Muñoz et al., 2008; Duan et al., 2011), possibly by having presented a physical barrier to pathogen development, as suggested by Baldwin et al. (1999). The fillers did not significantly contribute to reduce decay incidence.

3.5. Titratable acidity and soluble solids

The coated acerolas presented higher titratable acidity and lower soluble solids contents after 7 days of storage, when compared to uncoated fruit (Table 2). These findings indicate that the coatings have delayed the ripening process, corroborating previous studies evaluating effects of polysaccharide coatings on fresh produce (Dang et al., 2008; Sabularse et al.,2009; Duan et al., 2011; Maqbool et al., 2011). This ripening delaying effect is related to reduced respiration rates, which is expected from polysaccharide-based coatings.

3.6. Ascorbic acid

The ascorbic acid loss was reduced by all coatings, especially when nanocomposite coatings were used. The reduced ascorbic acid losses resulting from the presence of coatings are in agreement with previously reported effects of polysaccharide coatings (Chien et al., 2007; Maciel et al., 2004). The higher effectiveness of the nanocomposite coatings when compared to the unfilled coating is probably related to the improvement of the oxygen barrier by both cellulose whiskers (Sanchez-Garcia and Lagaron, 2010; Bendahou et al., 2011) and layered silicates (Petersson and Oksman, 2006; Bendahou et al., 2011), decreasing the rate of ascorbic acid oxidation.

4. Conclusions

Both reinforcements (cellulose whiskers and montmorillonite) improved water vapor barrier of stand-alone alginate–acerola puree films. When applied as coatings to fresh acerolas, the alginate–acerola puree film forming dispersions were effective to extend fruit stability by decreasing weight loss, ascorbic acid loss, and decay incidence, and by delaying the ripening process. When nanoreinforcements were added to the film forming dispersions, the ascorbic acid retention was further improved. The weight loss of acerolas was mostly reduced by montmorillonite-reinforced coatings. Moreover, montmorillonite maintained the red color of the coating anthocyanins, making the visual acceptance of coated acerolas similar to that of uncoated fruits. The anthocyanin stabilizing effect of MMT was ascribed to electrostatic interactions between flavylium cations and the clay platelets, and steric protection from degradation reactions.

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