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Effect of cashew gum-carboxymethylcellulose edible coatings in extending the shelf-life of fresh and cut guavas

Lucimara A. Forato[a], Douglas de Britto[b], Jackeline S. de Rizzo[a], Taís A. Gastaldi[a], Odilio B.G. Assis[a,*]

[a] Embrapa Instrumentação—Rua XV de Novembro, 1452, 13560-970 São Carlos, SP, Brazil
[b] Embrapa Semiárido—BR 428, Km 152, 56302-970 Petrolina, PE, Brazil

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ABSTRACT

Cashew gum (CG) and carboxymethylcellulose (CMC) based formulations have been evaluated as protective edible coatings on intact and cut red guavas. Samples were coated by dipping in aqueous mixtures of 1% CG and 1% plasticizer (glycerol) for CMC additions of 1 and 2% wt. The fruit was stored at ambient conditions (25–28 °C, 76.0 ± 12.4% RH), and loss of mass, color of pulp and peel, and texture were assessed. Magnetic Resonance Imaging (MRI) was used to visualize the internal structure decay of the intact fruits. Both coatings resulted in a reduction of mass loss, preserving firmness and delaying skin color changes. When comparing after 12 day storage, the mass loss in cut coated samples (CG plus 2% wt of CMC) was 38.5% inferior than that measure to uncoated references. At large, both coating formulations reduced water loss and changes in color of the cut surfaces. MRI analysis showed that tissue decay took place mainly near the peel or around the peduncle region between 8 and 12 days of storage at room temperature.

1. Introduction

Guava (*Psidium guajava* L.) is a tasty sweet fruit native from Central and South America. The guava tree is a very resistant culture (from the myrtle family—*Myrtaceae*), tolerating high temperatures and drought. It blooms continuously throughout the year, bearing at least two harvests annually (Morton, 1987). For many years guavas were exclusively associated with extractive exploitation. Recently however, in function of its proven nutritional properties (rich in vitamins A, C, iron, calcium and phosphorus), associated with ease of cultivation and a growing demand for manufactured products such as jams, jellies and juices, guavas are now cultivated in many tropical and subtropical countries.

One of the major drawbacks of fresh guava is that it perishes quickly. Guava is a climacteric fruit with elevated respiratory activity and high rate of ethylene production (Reys & Paull, 1995). This leads to a fast senescence process even under controlled refrigerated conditions (Srisvastava & Narasimhan, 1967). Various methods for postharvest conservation have been tried on fresh guavas, including the use of ionizing radiation to prevent microorganism proliferation (Silva, Correia, Moura, Maciel, & Villar, 2011); treatment by immersion in concentrated calcium chloride solution ($CaCl_2$) (Werner, Oliveira, Bona, Cavati, & Gomes, 2009) or calcium chloride associated with gibberellic acid ($C_{19}H_{22}O_6$) (Lima, Durigan, de Souza, & Donadon, 2003); polymeric packaging with controlled atmosphere (Singh & Pal, 2008a) and storage under 1-methylcyclopropene environment (Singh & Pal, 2008b). Additionally, edible coatings such as carnauba wax (Jacomino, Ojeda, Kluge, & Scarpare-Filho, 2003); cellulose emulsions (McGuirre & Hallman, 1995); starch and chitosan solutions (Soares et al., 2011), candelilla wax (Salinas-Hernández, Ulín-Montejo, & Saucedo-Veloz, 2010), milk-protein (Cerqueira, Jacomino, Sasaki, & Alleoni, 2011) and miscellaneous formulations based on gelatin, triacetin and lauric acid (Fakhouri, Batista, & Grosso, 2003) have been tested.

A potential biopolymeric material for edible coatings is cashew gum. Cashew gum (CG) is a non-toxic exudate polysaccharide obtained from the Anacardium occidentale tree. It is water soluble and can be transformed into transparent and resistant films by small additions of plasticizers and carboxymethylcellulose (CMC) (Britto, Rizzo, & Assis, 2012). The CG has a complex structure (Paula, Heatley, & Budd, 1998) and is reported to present antifungal and antibacterial properties against selected pathogenic and spoilage bacteria (Torquato et al., 2004). CG is typically used in popular medicines within Brazil such as cough syrup and as

* Corresponding author. Fax +55 16 21072902.
E-mail address: odilio.assis@embrapa.br (O.B.G. Assis).

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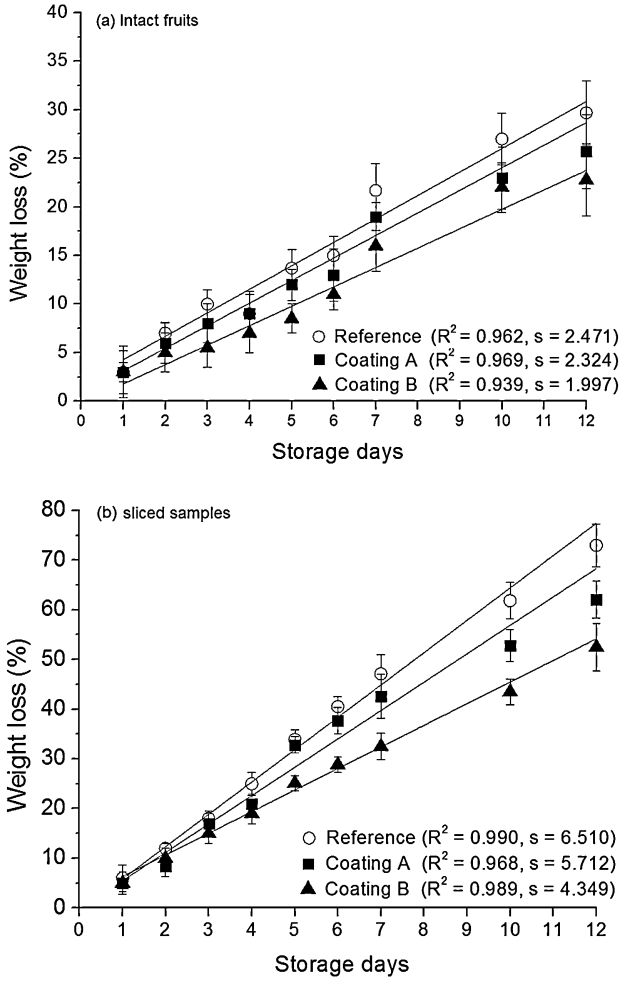

Fig. 1. Effect of CG-coatings of intact (a) and sliced guavas (b) on weight loss when storage at room temperature (25–30 °C and RH of 76.0 ± 12.4%). The error bars indicate the SD of thirty measurements.

thickening agent to stiffen the texture of broths and other liquid foods (Botelho, 1999). As an edible protective coating on fruits, CG with addition of plasticizers has been tested on apples by Carneiro-da-Cunha et al. (2009), with promising results in delaying general senescence.

This study aims to examine the effects of emulsions based on CG associated to CMC as edible coatings in prolonging the shelf life of intact and cut guavas stored at room temperature.

2. Materials and methods

2.1. Preparation of film-forming solutions

Crude natural exudate cashew gum was provided by Embrapa Agroindústria Tropical (CNPAT, Fortaleza, Brazil) and collected from cultivated *Anacardium occidentale* L. trees. Compositional analysis as reported by Paula, Heatley, and Budd (1998) revealed that the cashew gum structure is formed by complex chains of arabinogalactans rich in β-D-galactopyranose (72%) and α-D-glucopyranose (14%), with small fraction of arabinofuranose (4.6%), glucuronic acid (4.7%) and rhamnose (3.2%).

The CG was ground for composition homogenization and dissolved in distilled water under magnetic stirring at room temperature for 1 h. After another hour, in resting state, the precipitated gum was recovered (around 90% w/w) by suction filtration and the supernatant discarded. The recovered CG was dried in vacuum oven at 60 °C for 12 h and stored.

The coating formulations were then prepared by redissolution of the purified CG (1% w/w) in deionized water (pH 6.8). After complete solubilization, carboxymethylcellulose (CMC—Synth, Brazil) was separately added in two concentrations: 1 and 2% (w/v). Subsequently 1% (v/v) of glycerol (Gly—Synth, Brazil) was added to the solution as a plasticizer. The mixture was prepared in 500 ml beaker and moderately stirred with a magnetic bar at room temperature (~2 h) to assure homogenization.

Previous studies showed that pure cashew gum has no ability to form homogeneous films (Britto, Rizzo, & Assis, 2012), requiring the association with small amount of plasticizers and other polysaccharides such as CMC, which presents good filmogenic capacity, assuring the formation of a homogenous structures. For identification coating A refers to the composition made of 1% CG; 1% CMC; 1% Gly and coating B refers that of 1% CG; 2% CMC; 1% Gly.

2.2. Coating of guavas

Red guavas of *Kumagai* cultivar were acquired at the Terminal Market of Araraquara (SP, Brazil). The fruits were picked from the same lot and on the same day after delivery, reflecting at first, the same degree of maturity. The samples were sorted into similar size and mass (around 180–220 g) and without any physical damage or

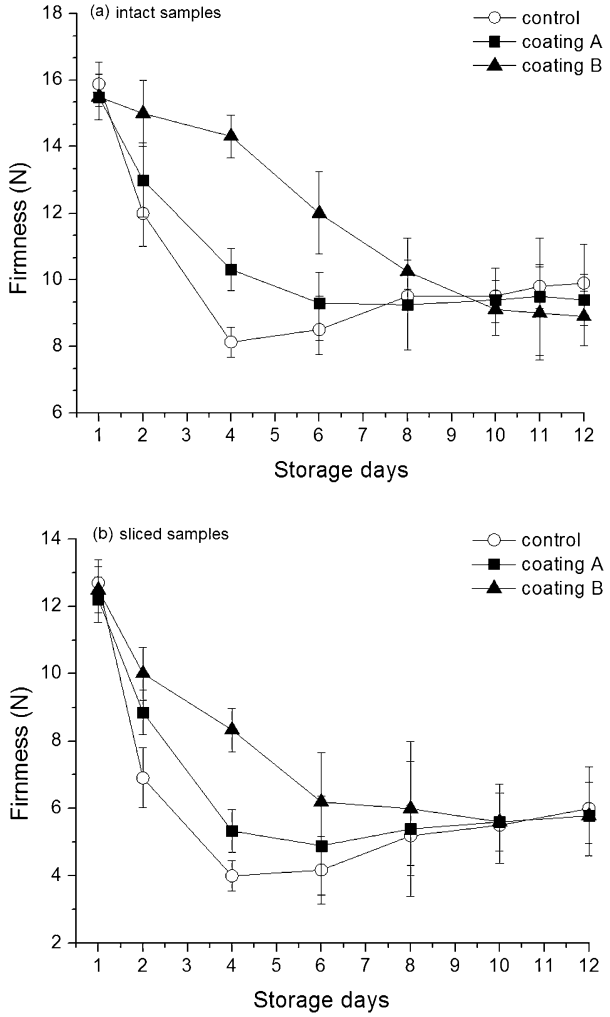

Fig. 2. Firmness of intact and cut guavas (uncoated and coated) during storage at room temperature and RH of 76.0 ± 12.4%. For sliced guavas the mesocarp region was probed. The error bars indicate the SD of nine measurements.

any visible signs of disease. After sanitization (immersion in an aqueous solution of sodium hypochlorite at 200 ppm for 10 min), the samples were dipped into the film formulations for approximately 5 s and the excess gel allowed to drain away. Batches of 60 fruits (intact and sliced longitudinally into two halves) were coated with each formulation and 60 other samples (intact and sliced) remained uncoated as control samples. The coatings were dried at room temperature. The samples (coated and uncoated) were separately stored under non-controlled conditions (room temperature between 25 and 28 °C and relative humidity of 76.0 ± 12.4%) whilst changes in the mass, color and texture were recorded daily. The storage at room temperature was chosen to simulate conditions commonly found in fruit sales in retail, as well as to accelerate postharvest ripening and senescence processes, becoming more evident differences between control and treated fruits.

2.3. Protective quality evaluation

Of each treated group (60 samples each), 30 fruits were preserved intact for weight loss, color evaluations and MRI, and 30 others left for destructive testing (firmness measurements). Samples were weighed using a digital Gehaka AG 2000 analytical scale (Gehaka Ltd., São Paulo, SP, Brazil) and mass loss was estimated as the average of individual weights. Relationships with the storage time were established using a linear regression model along with the R^2 value. The average gradients (slope) of each line were calculated by means of the first derivative expressing indirectly the rate of mass loss.

The firmness was assessed using a texturometer TA.XT Plus (Stable Micro System, UK) with a load cell of 50 kg and a speed penetration of 1 mm s^{-1}. The puncture test was performed using a stainless steel probe with 2 mm of diameter. The texture was measured as the maximum force (expressed in Newtons, 1 N = 0.1 Kg_F) required to achieve probe penetration of 8 mm at three different location in each sample. For whole fruits the texture was measured on samples with the skin and for cut fruits the mesocarp region was probed.

Color of intact and cut surfaces was evaluated using a colorimeter Chroma Meter CR-400 (Konica Minolta Sensing Inc., Sakai, Japan), and measurements were made at four locations for each sample at seven storage days. Ten samples in each condition were randomly chosen for measurements. The CIE—L*a*b* color system was used to evaluate the color changes. Peel and pulp color were evaluated by measuring the Hue angle (H = arctangent (b*/a*)), whereas 120 represents green, 90 yellow and 0° true red. The visual appearance of the fruits was also photographed.

Magnetic Resonance Imaging (MRI) was used to non-invasively investigate internal integrity of intact coated and uncoated guavas. The equipment was a Varian 2 T operating at a frequency of 85.53 MHz. A 'birdcage' single-tuned (^{1}H) probe with 14 cm of internal diameter was used. Longitudinal images were hydrogen weighted using a spin-echo pulse sequence with echo time of 20 ms and repetition time of 2.0 s. The field of view (FOV) was 15 cm × 15 cm and the matrix size was 128 × 128 pixels. The number of scans was 4 and slice thickness was 2 mm.

2.4. Statistical analysis

Three samples from each treatment were essayed resulting in a mean value of nine measurements each time. Relationships with the storage time were established by a linear regression model along with the R^2 value. Firmness and colorimetric data were subjected to statistical evaluation by one-way analysis of variance (ANOVA), following a completely randomized design considering significant difference at $p < 0.05$ using a Microcal Origin 8.0 software (OriginLab Co., Northampton, MA, USA).

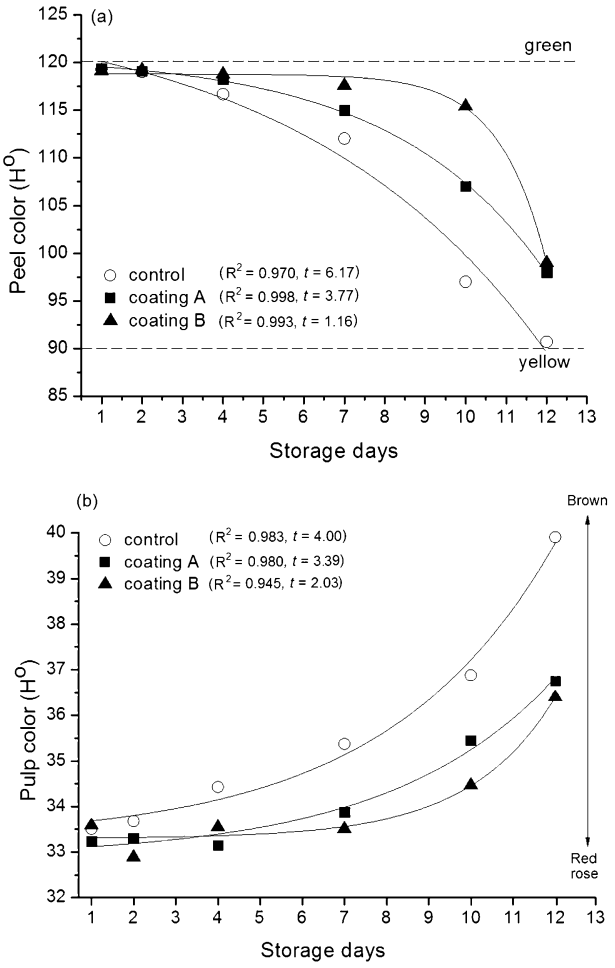

Fig. 3. Hue angle evolution in function of the storage time at room temperature and RH of 76.0 ± 12.4%: (a) peel and (b) mesocarp measurements. Each point represents the average of thirty measures.

3. Results and discussion

After solvent evaporation (10 min in air at room temperature) both formulations resulted in a highly transparent coating invisible to the naked eye. Weight loss measurements confirm that guavas underwent rapid mass decay when maintained at room temperature. Statistically no difference in loss was observed between coated and uncoated samples over the first four days. The protective effect is observed after this period when the values become statistically significant at 5%. This behavior was found both in intact and sliced samples (Fig. 1a and b).

The mass loss takes place mainly as dehydration, considering that losses due to respiration in intact guavas are very low and can be negligible as verified by Vazquez-Ochoa and Colinas-Leon (1990). The mass loss is more accentuated as expected for sliced samples, in which dehydration occurs easily through the cut surfaces. For both intact and sliced samples however, the weight loss shows a linear dependence to storage time. This data can be approximated to a linear model, such as ($y = a + sx$) which facilitates its interpretation. Such fitting gives s: the curve inclination (slope), that is physically interpreted as the rate of mass loss. In other words, the higher s the greater will be the dehydration as a function of time. The correlation parameter ($R^2 > 0.9$) confirmed the feasibility of the linear fitting.

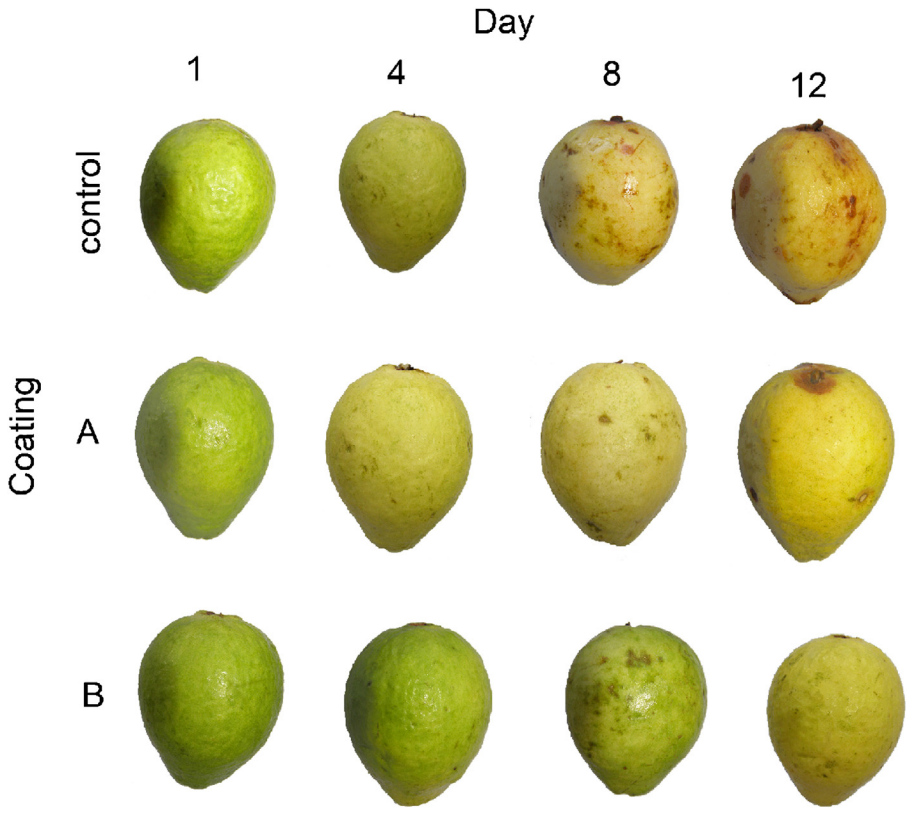

Fig. 4. Typical aspect of peel color during storage days at room temperature. Samples with no coating and coated with formulation A and B. Each point represents the average of forty measures. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

Accordingly to this analysis, the uncoated samples showed the fastest degradation rate and results indicate that the coating formulations act positively as a conservative agent, delaying the mass loss for both intact and sliced fruit (Fig. 1a and b). According to the time derivative s values, the non-coated control guavas lost around 2.5% of their mass daily, while for the coating A and coating B the losses were reduced to approximately 2% a day. The coating B behaves better, preserving by the end of the 12th day approximately 12% more mass than that measured on uncoated samples.

Interestingly for cut (sliced) guavas these relationships were more prevalent (Fig. 1b) where the coatings acted more efficiently as a barrier in reducing dehydration. According to the mathematic model, the non-coated sliced guavas lost around 6.5% of their mass daily while for coating A, the losses was reduced to 5.7%. More so was the reduction measured for coating B, where the losses drop to approximately 4% daily, i.e., a reduction proportional to 38.5% when compared to non-coated samples.

Weight loss associated with the fruit ripening also reflects as a progressive decline in flesh firmness. For both intact and sliced samples the reduction on firmness is evident and continuous with time, but it is notably preserved in the first 3–4 days of storage by the presence of CG coatings, (Fig. 2a and b).

Textural softening in guavas is strongly associated with the level of ethylene (Azzolini, Jacomino, Bron, Kluge, & Schiavinato, 2005), at room temperature the maximum of C_2H_4 production is reached in 4 to 6 days after harvesting (Brown & Wills, 1983). The consequence is that there is an increase in enzymatic activity on the cell wall components causing tissue flaccidity. For intact guavas stored at room temperature, a similar decay of firmness as presented here has been reported in studies carried out by Azzolini, Jacomino, Bron, Kluge and Schiavinato (2005) and Jacomino, Arruda, Bron, and Klunge (2008). When the samples are cut there is an additional stimulus for respiratory activity which increases CO_2 production. Such a condition induces the increasing C_2H_4 concentration, favoring the mesocarp firmness to fall more sharply as can be seen in the plots of Fig. 2b. The decay profile of firmness as measured in sliced guavas (uncoated samples) is in perfect agreement with the numerical data as collected by Souza, Cavalini, Jacomino, and Ortega (2009) under similar conditions. It can be observed that for both groups, intact or cut fruits, that after an initial softening by ripening, the process presents a loss of turgidity by dehydration resulting in an increase in firmness measurements. Such an increase after the softening period reflects the resistance to the probe penetrations due to the fibers resilience in a dry mass. All samples converge to similar firmness values, as observed in the graphics of Fig. 2, though such characteristics are delayed for coated samples in both intact and in sliced forms.

It is important to be remark that despite the untreated fruits have presented high dehydration along the first week, the experiment was extended for 12 days in order to determine the protective ability of coatings under the conditions adopted in this study. Additionally, it should be mentioned that for all samples the standard deviation increases with storage time because of the different evolution of each of essayed sample. Such variation among samples is natural and observed for several fruits during dehydration measurements (Camarena, Martínez, Ardid, Ramis, & Espinosa, 2005; Barriga-Téllez et al., 2011; Puerta-Gomez & Cisneros-Zevallos, 2011).

In Fig. 3 the Hue readings are plotted as a function of time. The yellowing tendency can be approximated to an exponential decay behavior according to a generic function $Y = Ae^{-(x/t)}$, where t indicates the temporal factor i.e., the rate (x/t) expresses the speed of the decay. All curves present $R^2 > 0.9$ indicating the goodness-of-fit for all conditions, allowing some interesting conclusions to be drawn.

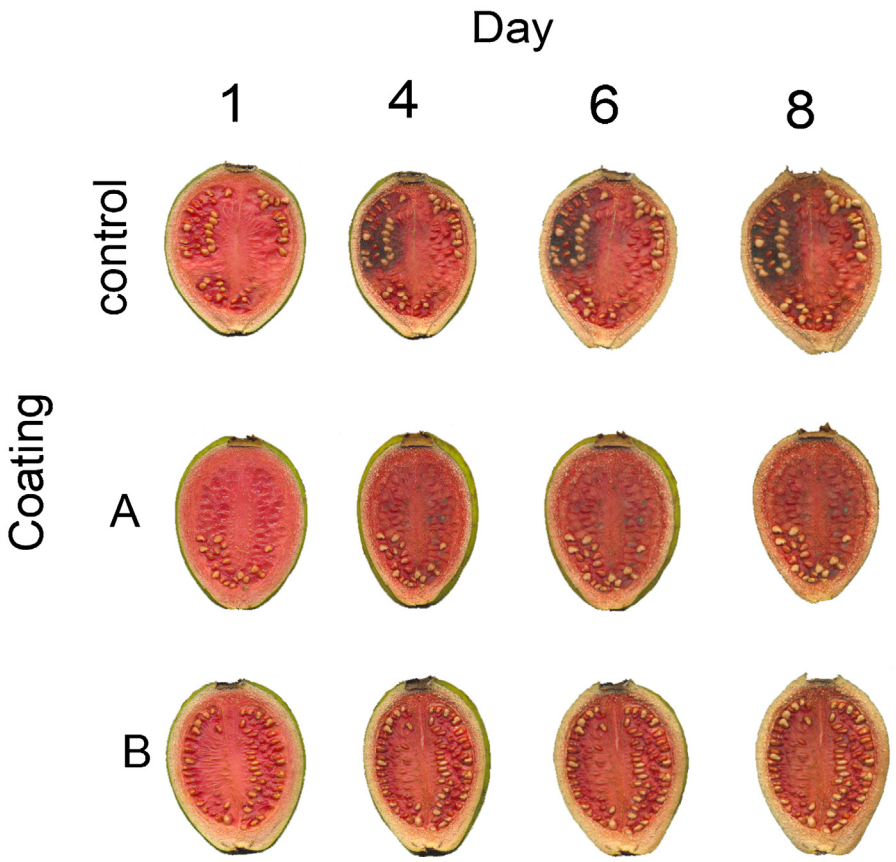

Fig. 5. Photographs showing the visual aspects of guavas pericarp as storage at room temperature. The dehydration and change of color is evident for all samples. For control slices the fungi proliferation is evident after the day 4, contributing to the darkening coloration as record by colorimetric measurements as displayed in Fig. 3(b).

From Fig. 3a, it can be observed that the peel on the whole fruit in the control samples suffers a sharp reduction in Hue angle values, continuously decaying during storage at room temperature. This result indicates a rapid change from a green quadrant (120°) toward a yellow one (90°). From the exponential adjustment the yellowing tendency can be assessed by comparing the t resulted from each fitted curve. It can be inferred that peel yellowing in the intact fruit is reduced by a half when coating A is used and delayed by around 6 times when coating B was applied.

The color change is a natural indicator of maturity. During the ripening process, the chlorophyll degrades exposing the carotenoids which are the main pigment responsible for most of the yellowish tinge. In general, the intensity of this yellowing is strongly dependent on the guava variety (Siqueira, da Costa, Afonso, & Clemente, 2011; González, Osorio, Meléndez-Martínez, González-Miret, & Heredia, 2011). Visual examples of peel color variation better illustrate the effectiveness of both coating formulations (Fig. 4).

Similarly, though less intense, the conservative effect of the coatings was observed on the cut samples according to color variation as illustrated in Fig. 3b. The freshly cut surfaces have a mean Hue angle value of $33.51 \pm 0.18°$, corresponding to a typical bright red-rose coloration. With time the Hue angles of all samples shifted to higher values (yellow quadrant), indicating that the color was becoming less red, shifting toward a dark or brownish coloration. The loss of initial vivid red color with a tendency to darken on the mesocarp is a natural occurrence where dehydration, enzymatic browning and fungi proliferation operate simultaneously. The coatings act as a barrier, mainly against excessive water loss and microorganism growth and mathematical analysis shows that the darkening on the uncoated surface occurs twice as faster than that measured on the surface coated by the formulation B.

Photography of the cut surfaces illustrates better effectiveness of both the coating formulations (Fig. 5). From these pictures, it is clear that the CG coatings also have an antifungal effect, showing that the fungi proliferation on the mesocarp/pericarp is reduced when compared with uncoated samples.

In order to assess the internal consistency of intact coated and uncoated fruits, MRI analysis was performed on random samples. By using MRI it is possible to monitor any minimal degradation progression and visualize the internal breakdown of a same set of samples with the advantage of not destroying it. The generated image is a combination of black, white, and shades of gray, contrast represents the intensity of the signal generated by ^{1}H atoms from water molecules. For guavas this corresponds to approximately 93% of the captured signals (Mattiuz, Biscegli, & Durigan, 2002).

Fig. 6 displays the MRI images acquired in 1, 6, 8 and 12 days of storage at room temperature. Up to the day 6, all images are quite similar, with a little whitening as the time passed by, indicating an increase in the free water content as the maturation proceeds. The increase of free water is a natural event resulting from the hydrolysis of starch into sugar plus water and can be accompanied by other metabolites such as oils and carbohydrates (Clark, Hocking, Joyce, & Mazucco, 1997). The increasing of free water can be also observed in pears with tissues affected by core-breakdown, differing from unaffected tissues (Wang & Wang, 1989).

From the fourth day onwards, black areas began to be observed in both the control and coating A samples (circulated in Fig. 6), mainly in the peduncle cavity region. Such a feature is attributed to the loss of ^{1}H signal from water, i.e., the water is absent in this

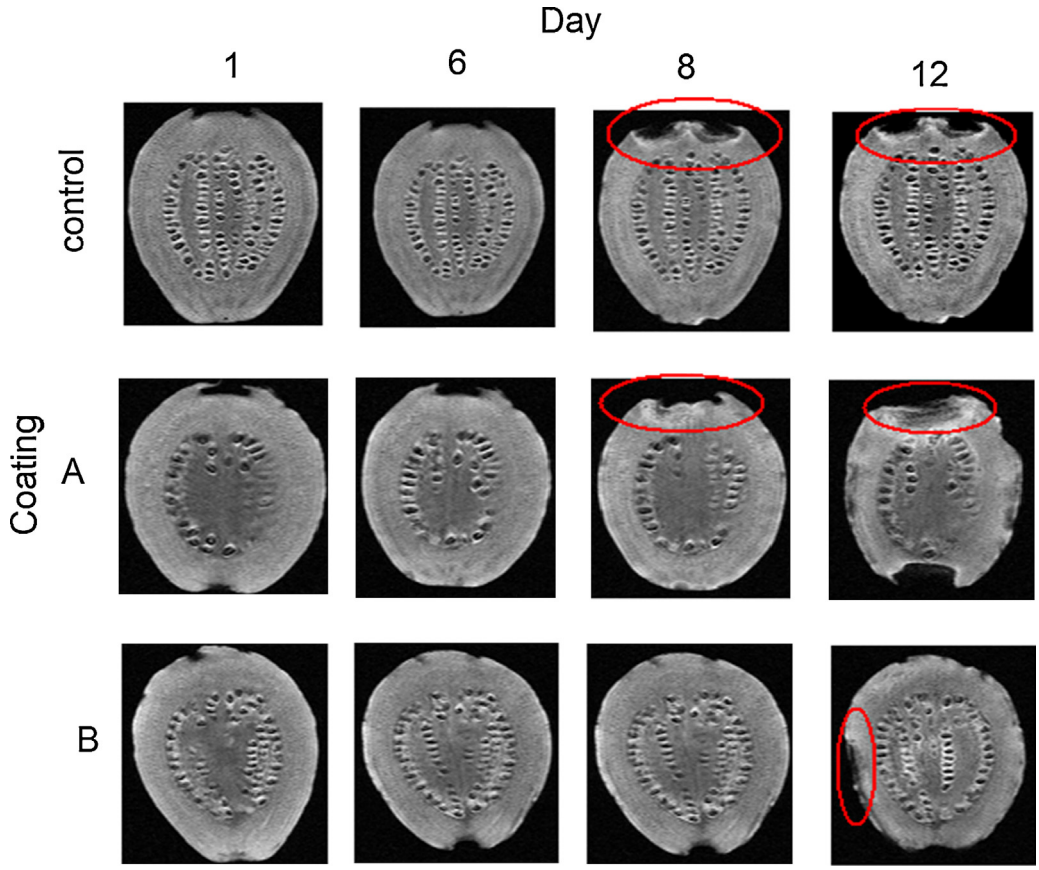

Fig. 6. Illustrative magnetic resonance images corresponding to uncoated (control) and coat guavas in different ripeness stages (days of storage at room temperature). The circulated regions highlighted the more intensive decay regions.

region (region of fast dehydration) or the presence of voids (Clark, Hocking, Joyce, & Mazucco, 1997; Burdon & Clark, 2001), pointing to tissue degradation or even a local necrosis. Such an observation is in agreement with the firmness data (Fig. 2a) in which after this period the loss of water becomes significant. It is worth observing that fruits with coating B do not show this effect, reflecting better integrity than the others samples.

After day 12, internal decays were found in all samples, although no extensive overall breakdown can be characterized. The tissue decay is more intensive around the skin and the penducle region, where intensive vascular activity takes place. It was observed that the internal decay, registered on coating B sample in day 12 does not reflect on its external appearance.

4. Conclusions

Coatings based on cashew gum (CG) associated with small additions of plasticizers (Gly) and carboxymethylcellulose (CMC) have been demonstrated as being effective in extending the shelf-life of guavas, both cut and uncut when stored at room temperature. The addition of CMC showed to play a relevant role in forming coatings which preserve the appearance by reducing color change (skin yellowing) and overall decay. These benefits are also observed in the pulp of the cut fruits. For the coatings an antifungal effect was also observed. Analysis by ^{1}H MRI in intact fruits has shown that despite external appearance, between 8 and 12 days of storage all samples had some internal degradation.

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