ANTIMICROBIAL EDIBLE COATING IN POST-HARVEST CONSERVATION OF GUAVA[1]

NILDA DE FÁTIMA FERREIRA SOARES[2], DANIELLE FABÍOLA PEREIRA SILVA[3], GEANY PERUCH CAMILLOTO[4], CRISTIANE PATRÍCIA OLIVEIRA[5], NEUMA MARIA PINHEIRO[6], EBER ANTONIO ALVES MEDEIROS[7]

ABSTRACT - This study aimed to develop an antimicrobial edible coating and to evaluate its efficiency in preserving guava. Guava samples were submitted to five treatments: without coating, coating of cassava starch, coating of cassava starch added with acetic acid, coating of cassava starch added with 1.0% and 1.5% chitosan. The following characteristics were analyzed: color of pulp and peel, texture, mass loss, total soluble solids, filamentous fungi and yeast counting. Peel green color of the fruits treated with antimicrobial coatings was preserved and the pulp color of all treatments changed from pink to intense red during the storage. Fruits treated with antimicrobial coatings presented lower mass loss, when compared with the control fruits. There was a decrease in the total soluble solid values of the control group during storage, whereas in the other treatments, the sugar content was preserved until the 8th day. Fruits treated with 1.0% and 1.5% of chitosan showed the lower filamentous fungi and yeast counting when compared with the control. The use of antimicrobial edible coatings contributes to the preservation of guava, reducing microbial growth and increasing its post-harvest life.

Index terms: *Pisidium guajava* L., starch, chitosan, postharvest.

USO DE REVESTIMENTO COMESTÍVEL E CONSERVAÇÃO POS-COLHEITA DE GOIABA

RESUMO - Objetivou-se desenvolver um revestimento comestível antimicrobiano e avaliar sua eficiência na conservação de goiaba. Os frutos foram submetidos a cinco tratamentos: sem revestimento, revestimento de amido de mandioca, revestimento de amido de mandioca com ácido acético e revestimento de amido de mandioca com 1,0% e 1,5% de quitosana. As análises realizadas foram: cor da polpa e casca, firmeza, perda de massa, sólidos solúveis e contagem de fungos filamentosos e leveduras. A coloração verde da casca dos frutos tratados com revestimentos contendo 1,0% e 1,5% de quitosana foi mantida, e a coloração da polpa de todos os tratamentos mudou de rósea para vermelho intenso durante o armazenamento. Os frutos tratados com revestimento contendo quitosana apresentaram menor perda de massa quando comparados aos frutos--controle. Houve redução no teor de sólidos solúveis para os frutos-controle durante o armazenamento, enquanto nos outros tratamentos, o teor de açúcar foi mantido até o 8º dia. Os revestimentos antimicrobianos apresentaram menores contagens de fungos filamentosos e leveduras quando comparados ao controle. O uso de revestimentos comestíveis antimicrobianos contribui na conservação de goiaba, aumentando a vida útil pós-colheita.

Termos para indexação: *Pisidium guajava* L., amido de mandioca, quitosana, pós-colheita.

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[2]Engª. Alimentos, PhD - Profª Adjunta – Departamento de Tecnologia de Alimentos - Universidade Federal de Viçosa, Av. PH. Rolfs s/n, Viçosa-MG, Brazil - 36570-000. E-mail: nfsoares@ufv.br. Autora para correspondência

[3]Engª. Agrª, DSc.- Pós-Doutoranda -Universidade Federal de Viçosa, Departamento de Fitotecnia 36570-000, Viçosa-MG, Brazil. E-mail: danieele@ufv.br

[4]Engª. Alimentos – Doutoranda - Departamento de Tecnologia de Alimentos -Universidade Federal de Viçosa, Av. PH. Rolfs s/n, Viçosa-MG, Brazil

[5]Engª. Alimentos – Professora -Universidade Estadual do Sudoeste da Bahia, Departamento de Tecnologia Rural e Animal, 457000-000, Itapetinga-BA, Brazil.

[6]Engª. Alimentos – Pesquisadora -Universidade Federal do Ceará, Centro de Ciências Agrárias, 60356-000, Fortaleza CE, Brazil.

[7]Eng. Agr. DSc.- Pós-Doutorando (Bolsista CNPq) – Departamento de Tecnologia de Alimentos – Universidade Federal de Viçosa, Av. PH. Rolfs s/n, Viçosa-MG, Brazil - 36570-000. E-mail: ebermedeiros@yahoo.com.br

INTRODUCTION

Guava is a highly perishable fruit that presents accelerated physiological processes. Its perishing effects are aggravated by storage conditions during postharvest processes (LIMA et al., 2002). Therefore, during post-harvest period, these fruits rapidly start senescence, a fact which prevents them from being stored for long periods. This is a very serious problem, because it hinders or even prevents the commerce of these fruits with distant markets, due to losses during transportation. Therefore, the application of postharvest technology is essential for guava market.

Films and edible coatings are an alternative for increasing shelf-life of fruits and vegetables, protecting fruits from humidity and oxygen effects, thus retarding their deterioration. Generally, films are elaborated from protein, hydrocolloids, fat or a combination of all of them. The advantage of compound films is the combination of the positive characteristics of each component (FAKHOURI et al., 2003), besides the fact that they are edible. Several authors have studied the use of edible coatings for the quality improvement of post-harvest vegetables (FAKHOURI et al., 2003; OLIVEIRA; CEREDA, 2003; PEREIRA et al., 2006; HENRIQUE; CEREDA, 2007; JÚNIOR et al., 2007; LEMOS et al., 2007; VILA et al., 2007). Besides regulating the metabolic activities of fruits, biofilms may become active packages as they interact with the product, preserving its quality and safety.

The replacement of the traditional chemical fungicides by natural antimicrobial substances is a favorable factor for the acceptance of agricultural products in the international market, due to the strict regulation on chemical use for food production (RAYBAUDI-MASSILIA et al., 2007).

Chitosan, which may be used itself as a biofilm, by itself or associated to other compounds, was very effective in inhibiting microorganisms during the post-harvest life of vegetables and fruits, and also in minimally processed food (LIU, 2007). Chitosan is a biopolymer of high potential to be used as an edible coating and active package, because it is non-toxic, and is able to form biodegradable films and prevent antimicrobial activity.

In this context, the objectives of this work were to develop an edible coating of cassava starch and chitosan to be used on the surface of guava and to evaluate its preservation efficiency through microbiological and physicochemical analyses.

MATERIALS AND METHODS

2.1 Preparation of fruits

The 'Pedro Sato' guava (*Pisidium guajava* L.) variety was harvested and transported to a packaging house, where the fruits were selected according to their size and maturation stage, and those with physical injuries and physiological disturbances were discarded. Then, the fruits were kept in cardboard boxes and transported to the Packaging Laboratory at the Food Technology Department, in the Federal University of Vicosa, UFV, Vicosa, MG, Brazil. The guava samples selected weighed around 150.04 ± 12.04 g. The fruits were then washed in water, rinsed in Sumaveg® solution (Diversey Lever) (200 mg L^{-1} of total residual chlorine) for 10 min, washed in a solution of 3 mg L^{-1} residual chlorine and left at room temperature to dry. Next, the fruits were stored at 10 °C until they were used in the experiment.

2.2 Preparation and application of coatings

The coatings were prepared using cassava starch and chitosan (PADETEC – Brazil), with a disacetilation degree higher than 85%.

To prepare coating treatments, aqueous solutions containing 2.5% of cassava, 2.0% of glycerol and chitosan (1.0% and 1.5% w/w), previously dissolved in 0.4% glacial acetic acid (w/w) were heated at 70 °C until the starch gelatinized. Besides, coating treatments without chitosan and cassava starch added with only 0.4% glacial acetic acid were prepared, as shown in Table 1.

The guava samples were immersed into different coatings for 3 min and dried at 25 ± 2 °C for 3 h. The fruits were stored at an average temperature of 22 ± 2 °C and relative humidity equal to 62 ± 6%, for 12 d.

The guava samples were analyzed on day zero and every 4 d, during 12 d.

2.3 Analysis

The peel color evaluation was carried out using a colorimeter (Color Reader CR- 10 Minolta), and the results expressed in b* parameter. The pulp color was quantified through the equation $\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{1/2}$. For the peel color, two readings were performed in each diagonally opposed side of the fruit. For the pulp color, the readings in triplicate were conducted in the placenta region.

The fruit strength was evaluated through a compression test, using an equipment for universal mechanical testing (Instron, model 3367). For this analysis, the guavas were cut in half in the longitudinal direction. A 15 mm and a 1 kN weight probe were used. The test velocity was equal to 1 mm s^{-1}. The results were given by the maximum average compression (N) of two fruits.

The mass loss was determined by weighing fruits in a semi-analytical scale, every day during the 12 storage days. The results were expressed in percentage of mass loss.

The total soluble solid (SST) content of the guava pulp was determined using manual refractometer (Atago), with reading between 0 and 32° Brix.

The Titratable Acidity was determined by titration, using NaOH 0.1 N and expressed in percentage of citric acid (BRASIL, 2005).

The coating antimicrobial efficiency on guava was evaluated by the counting of the filamentous fungi and yeast. For the microbiological analysis, samples containing 25 g of 0.5 cm thick peel were homogenized in 225 mL of peptonated water at 0.1%, in sterile bags and agitated in a Stomacher for 120 s. From the broth, subsequent dilutions were conducted to be used for the plate method in Agar Dextrose Potato (BDA). The results were given in CFU g^{-1} of peel. The coated guava was analyzed after each treatment in time zero.

The experiment was conducted three times and two fruits per repetitions, in an entirely randomized design, in factorial scheme, with time and coating type as factors under study. The regression models were chosen based on the significance of the regression coefficients at 5% probability. The statistical analyses were conducted using the Statistical Analysis System (SAS), version 9.1.

RESULTS AND DISCUSSION

One of the main characteristics to indicate the maturation process of fruits in the post-harvest phase is the peel color. The loss of the green color of the peel is due to a break in the chlorophyll molecular structure, involving the chlorophylase enzyme. An increase of the enzymatic activity is generally associated with ethylene production during ripening (BASSETO et al., 2005).

Higher b values indicate that the peel color is more yellowish, showing that in treatments without chitosan, ripening and loss of pigment occurred faster. There was a significant effect ($p<0.05$) on fruits treated with antimicrobial coatings, whose peel color was remained green for the 12 d of storage, while the peel color of the control group changed from green to yellow in only 4 d. On the 8th day of storage, the fruits started to present a brownish color, due to spoilage (Figures 1 and 2). Similar results were obtained for apples and strawberries (RAYBAUDI-MASSILIA et al., 2007). Scanavaca Junior et al. (2007), studying the "Surpresa" mango variety, treated with 0, 10, 20 and 30 g L^{-1} of cassava starch coating, also reported that the fruits treated with 0 g L^{-1} of cassava became yellow in 12 d, and treatments using 20 and 30 g L^{-1} of cassava starch changed from green to greenish-yellow during the same period.

The variation of pulp color (ΔE) increased as time passed, in all treatments, including the control group treatment, indicating the ripening of fruits while stored. According to Watanabe et al. (2011), color change during fruit maturation occurs due to degradable or synthetic processes, and it is considered one of the main judging criteria for the ripening of fruits and vegetables.

The pulp color in the placenta region was pinkish when the fruits were harvest, and it became intense red after ripening for all treatments (Figure 3A). However, in fruits without coating, the pulp color degradation was faster than in other treatments, reaching ΔE value of 152.54, while chitosan treatments had ΔE values of 34.05 and 15.52 for coating 3 and 4, respectively, on the 12th day of storage. In cassava concentrations associated with chitosan, the ΔE values were lower.

The fruits submitted to the chitosan treatment presented color change only in the placenta region, once their epidermal color was the same as postharvest fruits.

Fruits submitted to treatments without coating (control group) presented softer pulps during storage, in comparison to the fruits submitted to other treatments (Figure 3B). The fruits coated with chitosan (1.5%) showed higher strength force, keeping pulp firmness during all the storage period. Similar results were obtained by Pereira et al. (2006), who studied the ripening of the Formosa papaya variety with edible coating of cassava starch; the loss of strength of the fruits treated with 1% and 3% was lower than that of the control treatment.

The higher strength presented by the coated fruits will guarantee better resistance against mechanic injuries during the post harvest processes, and consequently, higher durability.

Fruits without coating (Figure 3C) presented higher values of mass loss, reaching the 8th day of storage with a loss higher than 20%. While in fruits coated with 1.5% chitosan, mass loss was 11.8% during the same storage period.

In studies on the Formosa papaya variety with coating of cassava starch, Pereira et al., (2006) verified that the treatments did not present significant effects on the mass loss variable, although absolute values presented a lower loss as cassava starch concentration in the suspension increased, which may be due to the reduction of fruit water loss, caused by the increase in coating thickness.

According to Oliveira and Cereda (2003), acceptable values of mass loss for guava vary between 10% and 15%. Therefore, control fruits were not appropriate for consumption on the 4th day of storage. As to the treatments, the control group presented a decrease in the total soluble solid values during the experiment, while the other treatments were more effective in controlling the sugar content until the 8th day of storage (Figure 4A). Bashir e Abu-Goukh (2003) detected 5 and 8% of sugar reducer content, at the climacteric peak, in white and red guava pulps, respectively. Besides, these authors observed an increase in sugar reducer content until the fruit reach the climacteric peak, which is followed by reduction. According to Hojo et al., (2007), soluble solid content of 'Pedro Sato' guava pulp varied from 6.3 to 9.7%. The fruits used in this experiment presented SST values of around 12.5%.

Acidity is one of the criteria used to classify fruits by its flavor. For Pedro Sato cultivar, acidity values from 0.2 to 0.9 g of citric acid per 100 g of pulp give guava a moderate flavor and good acceptance for *in natura* consumption (HOJO et al., 2007). In vegetables, this characteristic is attributed mainly to organic acids inside cell vacuoles, both in combined and free forms. Organic acid content, with a few exceptions, decreased as fruits ripened, due to its use as a subtract in the respiratory process or sugar conversion (PEREIRA et al., 2006).

For all treatments, fruits presented a decrease in the titratable acidity content (Figure 4B) during storage. Fruits from the control group reached higher titrated acidity values (around 0.45%) if compared to fruits coated with chitosan (1.5%), which reached the value of 0.37% at the 12th day of storage. These results agree with those found by Oliveira and Cereda (2003), who found a decrease in ATT values for peaches submitted to treatments with different coatings and stored under room conditions (27.2 ± 3 °C and 70 ± 11% UR) for 12 d. Peaches without coating presented acidity peak on the 12th day of storage, due to the advanced senescence stage.

According to the variance analysis, the interaction between coatings and time was significant ($p<0.05$) on the filamentous fungi and yeast counting. Figure 4C shows the statistical adjustment for the counting of the filamentous fungi and yeast (Log CFU g^{-1} of the peel) of guava submitted to different treatments according to time (days).

The initial counting of the filamentous fungi and yeast on peels of guava samples was 1.11 Log CFU g^{-1}. Until the 4th day, the control fruits and those involved with different coatings presented similar counting.

The coatings containing 1.0% and 1.5% of chitosan presented higher antimicrobial efficiency on filamentous fungi and yeast inhibition. After 12 d of storage, the coated guava samples showed a reduction of 1.98 and 3.01 logarithms cycles, respectively, in comparison to the control group. According to Botrel et al. (2007), the effect of chitosan on fungi activity is due to possible alterations in the membrane functions, through interactions with the chitosan electronegative surface, leading to changes of permeability, metabolic disturbances and death of the cell.

Coatings 1 and 2 also inhibit the growth of filamentous fungi and yeast in guava, during the 12 d of storage, showing a reduction of 2.41 and 1.95 logarithms cycles, respectively. These results showed that coatings free from antimicrobials contributed to retard filamentous fungi and yeast growth in *in natura* guava.

Durango et al. (2006) reported that starch-based edible coating from dioscorea added with 1.5% chitosan presented antimicrobial activity on filamentous fungi and yeast for minimally processed carrots, during 15 d of storage under 10 °C.

Botrel et al. (2007), while studying the antimicrobial effect of starch-based edible coating from cassava added with 0.5% chitosan for minimally processed garlic, observed its efficiency on filamentous fungi and yeast, showing a reduction of 1,98 logarithms cycles in relation to non-coated garlic, after 25 d of storage under 10 °C.

FIGURE 1 – Guava submitted to different treatments: (A) Control – without coating; (B) Coating 1; (C) Coating 2; (D) Coating 3; (E) Coating 4; after stored for 12 days.

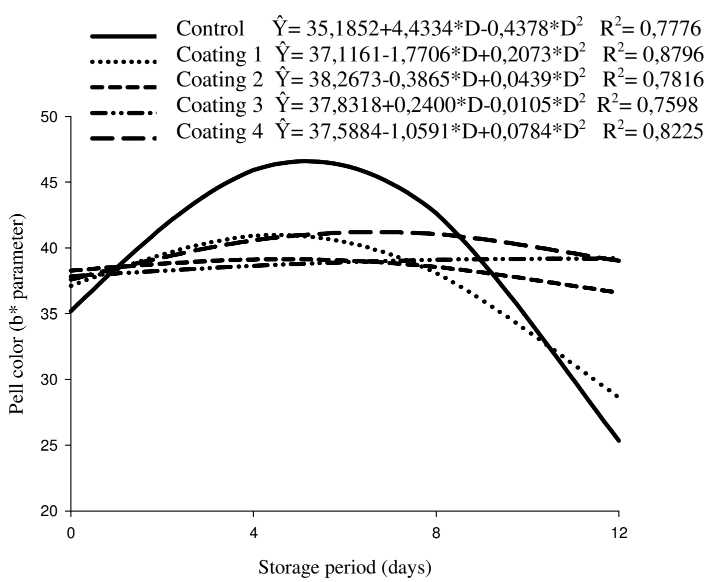

FIGURE 2 – Coating effect in b coordinate in peel color of ‘Pedro Sato’ guava, stored at 22.12 ± 1.98 °C during storage.

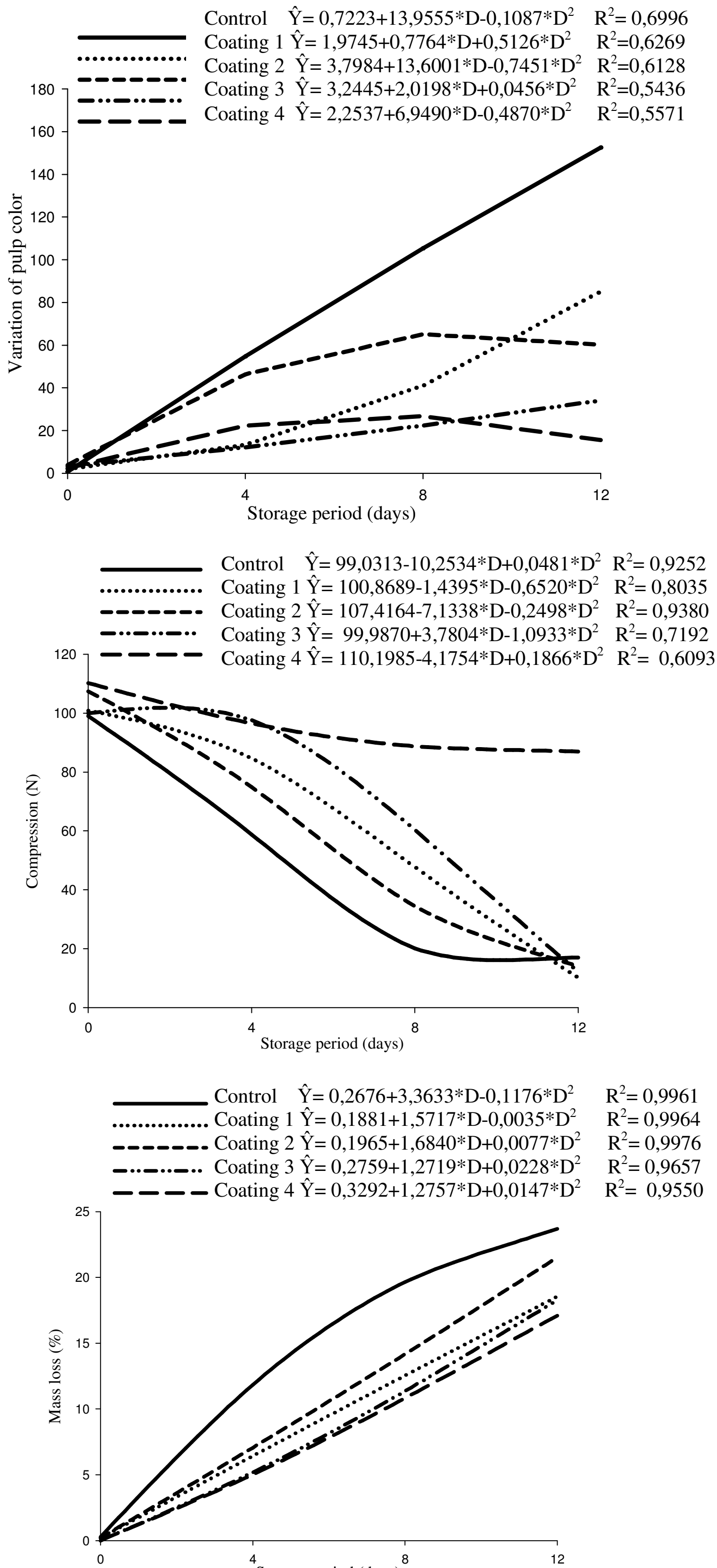

FIGURE 3 – Coating effects on pulp color difference (ΔE) (A), effects the compression (N) (B), fresh mass loss (C) of 'Pedro Sato' guava, stored at 22.12 ± 1.98 °C during storage.

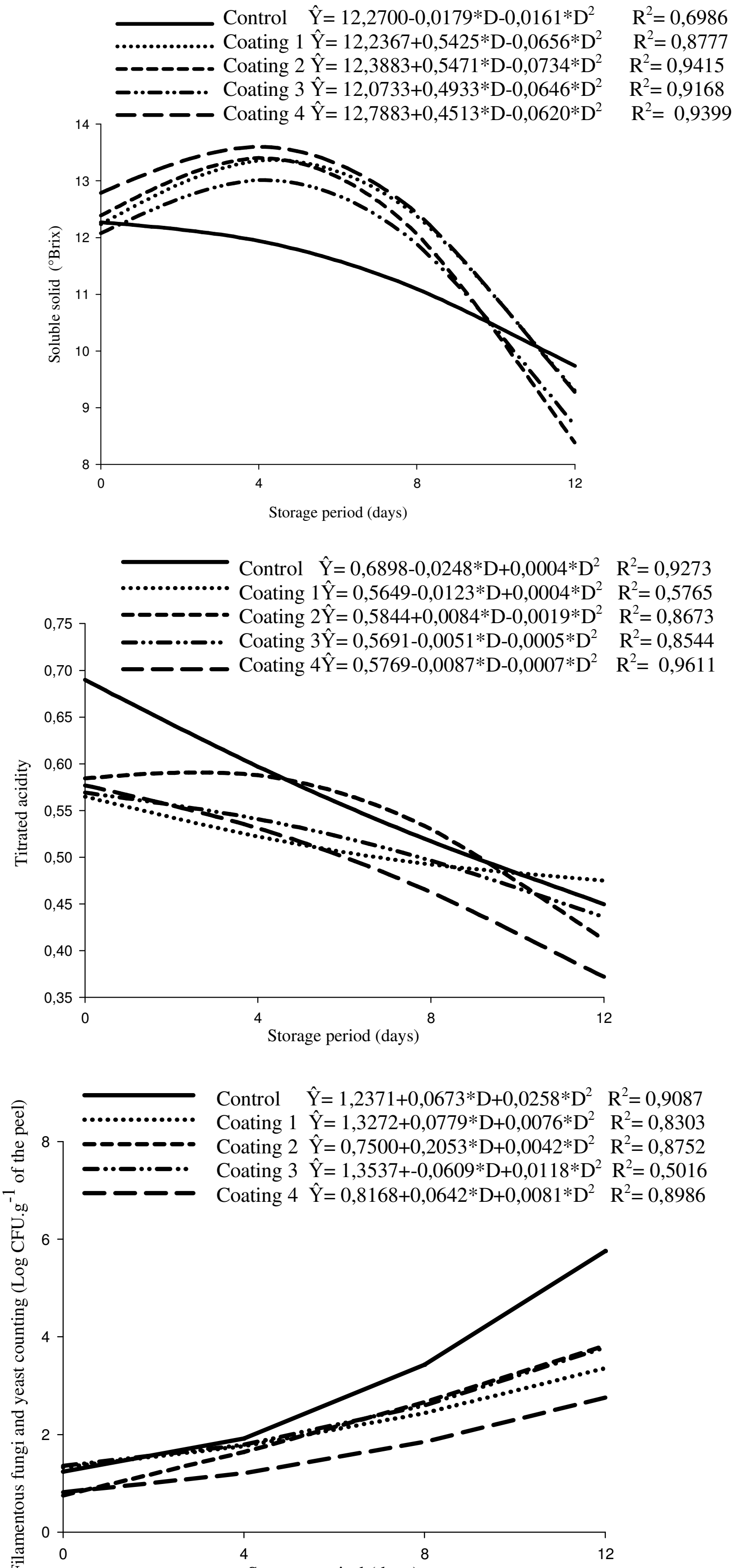

FIGURE 4 – Coating effects on soluble solid (A) titrated acidity (B) and effect on filamentous fungi and yeast growth on the peel (C) of 'Pedro Sato' guava, stored at 22 ± 1.98 °C during storage.

CONCLUSION

The use of edible coating was efficient to extend guava shelf-life, keeping the green color of its peel and the pink color and strength of its pulp. Coatings of cassava starch added with chitosan presented an antimicrobial effect on filamentous fungi and yeast. The use of edible coating in association with natural antimicrobials is a good alternative to increase fruit shelf-life.

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