Effects of carnauba wax and chitosan bilayer edible coating on shelf life of fresh-cut apple

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Abstract

Edible coatings can be an alternative to extend shelf-life and preserve quality of fresh-cut fruits by forming a barrier that avoids physical and microbiological damages. Such coatings can be formed by individual or multiple layers. The main aim of this study consisted in the evaluation of the effect of a bilayer coating (carnauba wax and chitosan) on the quality of fresh-cut apples. Chitosan and carnauba wax were characterized, individually and in bilayer format, by Attenuated Total Reflectance (ATR). The tests were performed on apple cv. Gala, sliced and sanitized, in five different treatments: (T1) uncoated, taken as control; (T2) Ascorbic acid solution at 1%; (T3) Chitosan at 1.5%; (T4) Carnauba wax at 0.5% and (T5) Bilayer – (Chitosan 1.5% + Carnauba wax 0.5%). Physicochemical and microbial analyses were carried out every two days along 10 days storage at 5°C. Sensorial analyses were conducted on the fifth and tenth day. ATR revealed no interaction between main functional groups of carnauba wax and chitosan groups on the bilayer formations and no differences in firmness and weight loss were found among samples during the storage time. Color measurements confirmed that coated slices became dark faster than uncoated samples. Concerning microbiological analyses (total coliform microorganism and *E. coli*), slices coated with both chitosan and carnauba+chitosan bilayer, had the bacterial growing rate reduced. In sensorial analyses, panelists pointed their preference for apple slices treated with carnauba and also for bilayer coating, choice probably related to fruit appearance.

Keywords: fruit quality, physic-chemical, microbiological analyses, sensorial analyses

INTRODUCTION

Apple is one of the most consumed fruits worldwide, either in natura or in fresh cut or processed into juice. The main apple producers are China, Europe Union, USA, Turkey, Iran, India, Russia, Chile, Ukraine, and Brazil, in a total worldwide harvest estimated at 68.64 million metric tons for the 2018/2019 season (Agrianual, 2019). Fresh-cutting is one alternative that can facilitate consumption and decrease wastes. However, due to the slicing and peeling processes, apple becomes more perishable requiring technologies that increase the product shelf-life.

One alternative technology suitable to keep shelf-life quality on the fresh cut products is the applications of edible coatings (Guerreiro et al., 2017). Edible coatings act as a barrier avoiding physical, chemical and microbiological damages (Embuscado and Huber, 2009). Lipid-based compounds, such as Carnauba wax has been widely used as a coating former, with good action in reducing the loss of water and the fruit surface abrasion, improving the mechanical integrity and controlling the internal gas composition (De Freitas et al., 2019; Lin and Zhao, 2007). Moreover, Gonçalves et al. (2010) reported that carnauba wax can impart antimicrobial protections as observed on coated nectarine and plum. On the other hand, chitosan is a polysaccharide obtained from the alkaline deacetylation of chitin that have been extensively used in fruit coatings, especially fresh cut apples (Pilon et al., 2015). Chitosan is reported as having a wide antimicrobial activity against several groups of foodborne pathogens, mainly bacteria and fungi (Vásconez et al., 2009; Garrido Assis and Britto, 2011).

Edible coatings can be formulated with pure compounds (Motamedi et al., 2018) or by several compounds, in layer-by-layer method resulting in multilayer coatings with high stability under different environmental stress (Mantilla et al., 2013; Acevedo-Fani et al., 2017). Carnauba wax associated cassava starch was evaluated on apple slices with positive results on reducing respiration rate and increasing water vapor resistance (Chiumarelli and Hubinger, 2012). On blueberry fruit, coatings of chitosan with aloe vera resulted in effective reduction over the lag mold contaminations and loss of water, when compared to uncoated fruits (Vieira et al., 2016). Furthermore, the association of chitosan and carnauba wax, as previously tested on strawberries, showed to have positive action in preserving firmness and reducing weight loss (Toliba et al., 2014).

Therefore, the hypothesis here is that the benefit effects of both carnauba and chitosan, in bilayer coating formation, can be adequately applied to enhance the conservation of fresh-cut apples. So, the main aim of the present study was to form such edible bilayer coatings, made of carnauba wax and chitosan, and evaluate their effect on the quality of fresh cut apples, along ten days storage under refrigeration.

MATERIAL AND METHODS

Material

1. Chitosan solution

Chitosan gel (from Sigma-Aldrich, medium molar weight), was prepared by dissolving 1.5 g into 1 L of 2% citric acid aqueous solution under constant stirring for 12 h. The solution was heated to 40°C, and the pH solution measured as 4.1 (Pilon et al., 2015).

2. Carnauba wax solution

The oil phase was composed of carnauba wax type 1 (8 to 18%, wt/v), provided by Pontes Ltda (Parnaíba, PI, Brazil), and palm oleic acid (2.6 to 6%, wt/v) from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The water phase was prepared by mixing ammonium hydroxide (1 to 3%, wt/v), and dimethylpolysiloxane (0.02 to 0.1%, v/v) (both from Sigma-Aldrich) with deionized water (71 to 89 wt/v%). The oil phase was heated to 105°C in an open reactor under mechanical stirring at 100 rpm during 10 min. Twenty to 30% of the water phase, heated to 100°C, was slowly added forming an oil/water (O/W) system. The emulsion solution remained under stirring at 150 rpm for 20 min and cooled down to 24°C, in water bath. The procedure followed the ammonium emulsification methodology, as described by Hagenmaier and Baker (1997), with slight modifications.

Methods

1. Sample characterization: attenuated total reflectance (ATR).

The chitosan and carnauba wax were characterized individually and in bilayer form by attenuated total reflectance (ATR) technique, using a Bruyer (Germany) Vertex 70 Medium Infrared Fourier Transform (FTIR) Spectrometer. Samples used for spectroscopy analysis were prepared as films by casting the solutions on petri dishes and dried for 24 h. The analysis aim at identify any possible interaction between the functional groups of chitosan and carnauba wax in the formation of a bilayer.

2. Fresh-cut coating.

All tools used in the process were sanitized with liquid detergent, washed with distilled water and then disinfected with 70% alcohol. PET (polyethylene terephthalate) packaging for storage were washed and disinfected with hypochlorite solution. The storage chambers were sanitized with quaternary ammonia and sample preparation was conducted at 5°C. Apples were cut into 6 equal slices and the seeds removed. The slices were sanitized in a chlorine dioxide solution for 3 min. The samples underwent the following treatments: T1 – uncoated slices (control); T2 – slices immersed in 1% of ascorbic acid (AA) for 3 min; T3 – slices

immersed in AA for 3 min, drained for equal time and then coated by spraying with 1.5% chitosan; T4 – apples immersed in aa and coated with 0.5% carnauba wax by spraying; T5 – slices immersed in aa 1% for 3 min, drained for equal time, then chitosan 1.5% was applied and after 30 min sprayed carnauba wax at 0.5% to form a bilayer coating. All samples were packed separately in polyethylene packages, containing 6 slices of each treatment and stored in a cold room at 5°C. The analyses were performed every two days along 10 days of storage. All assays were carried out in triplicate.

3. Physicochemical analysis.

To determine the apples flesh colors, a colorimeter Konica Minolta model CR400 was used, in L*, a*, b* system of color. Six measurements were made for each treatment, in two replicates for each slice.

Firmness was evaluated using a Texture Analyzer TA.XT.Plus Micro System texturometer, with cylindrical probe of stainless steel with a diameter of 4 mm (model P/4). Fruit penetration velocity was 1.0 mm s^{-1} with a 5 mm depth. Three measurements were considered in each slice. The losses of mass were determined by daily weighing of the packages in a digital balance, model AS 2000C.

4. Microbiological analyses.

The microbiological analyses were essayed on six slices per package. The slices were washed in 1% sterile peptone water and taken as dilution 100. The dilution 10^{-1} was obtained by pipetting 1 mL of the initial washing solution into 9 mL of sterile peptone water (1%), and from this the dilution 10^{-2} was similarly prepared. After the incubation time for each microorganism, the colonies were counted. The results were expressed in log UFC g^{-1} considering the arithmetic means of the triplicate multiplied by the respective dilution value.

For psychotropic aerobic microorganisms, 100 µL of diluted culture were incubated in Petri dishes and incubated at 35°C for 48 h for subsequent counting of the colonies.

The total coliform microorganisms and *E. coli* strains counting were performed in Petrifilm™ 3M after inoculation with 1 mL of each dilution, according to the manufacturer's recommendations. The plates were incubated at 35°C for 24 h before colonies counting.

A Tecra TM 3M kit was used to detect the presence of salmonella, via visual coloration changes.

5. Sensorial analysis.

Sensorial analysis was performed at two moments of the experiment. One on the fifth day and other one on the tenth day of storage. Thirty untrained panelists, evaluated apple's slices. Panelists were asked to rate samples for color, visual firmness and overall appearance on a 5-point category scale (1 – dislike extremely, 2 – dislike considerably, 3 – neither like nor dislike, 4 – like considerably and 5 – like extremely). Fruit samples were presented with a three digit code following a randomized design.

6. Statistical analysis.

Statistical analysis was performed using univariate parametric analysis of variance with a fixed effect factor (treatment), at five levels and Duncan's or Games-Howell's multiple comparison test. The parameters evaluated were: i) Initial value for weight loss and color (obtained from the linear regression adjustment for each replicate); ii) Weight loss and color changes of rate, in the period as calculated from the linear regression adjustment for each replicate; iii) Average values of texture evaluation (adjustments were not significant for most of the repetitions); iv) Rates of microbiological growth were obtained by the method of differential equations, as described by Baranyi and Roberts (1994). For treatment of sensorial data, the non-parametric ANOVA was used with five levels and multiple comparisons test of Kruskal-Wallis. The significance level set for all analyzes was 5%, using the software SSS vs. 20, Statistica version 7 and DMFIT 3.5.

RESULTS AND DISCUSSION

Sample characterization: attenuated total reflectance (ATR)

The infrared spectra of carnauba wax, chitosan, and bilayer were evaluated to verify possible interactions between functional groups for carnauba wax and chitosan molecules after bilayer preparation. Most of the main peaks of chitosan and carnauba were preserved after the bilayer formation indicating few or unidentified interactions between mainly functional groups of both compounds, assuring non-significant losses of individual activities. Small changes, however, are observed in the OH region (around 330 cm^{-1}) and 1000 cm^{-1} corresponding vibrations associated to -C-O-C- stretching in glycosidic linkage of chitosan structure and aromatic groups of carnauba, as identified in Figure 1. Such bands shift suggests the formation of intermolecular hydrogen bonds between compounds which guarantee the mechanical stability of the bilayer.

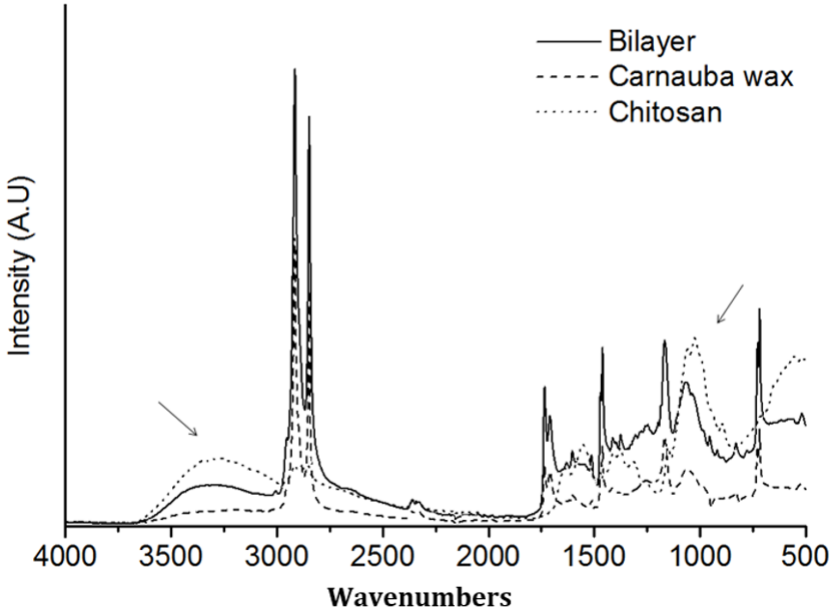

Figure 1. Infrared spectrum of carnauba wax, chitosan and bilayer.

Physicochemical analysis

1. Flesh color.

There were not significant differences among the treatments for color analysis just after coating (Table 1). It was observed small differences in initial parameters after coating in treatments, as read for a* value and hue. For a* value, T2 (ascorbic acid) and T4 (Carnauba individually), slices tend toward a green color than the other samples. T4 (Carnauba wax 0.5%) showed the highest value for hue and T3 (Chitosan 1.5%) the lowest, indicating a tendency for more yellow color resulted from the T3 coating. This difference may be related to citric acid used for chitosan preparation. No differences were found for L* and Chroma, besides ascorbic acid promoted a decreasing of enzymatic browning, which is however, rapidly degraded.

For daily rate changes (Figure 2) it was observed a daily increase of L* values for T5 (bilayer), followed by T3 (Chitosan 1.5%). T1 (ascorbic acid) resulted in lowest changes. Negative L values indicate surface darkening, therefore control samples did not show intensive color changes. Hue and chroma have similar behavior, with high values mainly related to coated treatments, particularly for the bilayer treatment indicating high color saturation.

Table 1. Color measurement at day 1 after postharvest treatments, for parameters a *, b *, L, chroma and hue.

Variable	T1	T2	T3	T4	T5
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
a*	-3.8±0.4 b	-4.6±0.3 a	-3.3±0.5 b	-4.6±0.4 a	-3.9±0.1 b
b*	28.5±0.9 a	27.5±1.2 a	29.0±0.8 a	26.4±1.0 a	27.5±1.1 a
L	81.1±0.5 a	81.3±0.7 a	79.9±0.9 a	80.5±0.5 a	80.0±0.7 a
Chroma	28.8±0.9 a	27.8±1.2 a	29.2±0.8 a	26.8±0.9 a	27.8±1.1 a
Hue	97.5±0.9 ab	99.5±0.7 cd	96.4±0.7 a	99.9±1.3 d	98.1±0.1 bc

T1=control; T2=ascorbic acid 1%; T3=Chitosan 1.5%; T4=0.5% carnauba wax; T5=Chitosan 1.5% + Carnauba wax 0.5%. Means followed by the same letter on the line do not differ at 5% probability by Duncan test.

Weight loss and firmness measurement

No significant differences were measured for weight loss and firmness among treatments. For fresh cut apple, Pilon et al. (2015) found no differences in firmness in control and chitosan treated samples, in agreement to our results. Although previous studies also report that carnauba wax is effective in maintaining firmness and reducing weight losses (Jo et al., 2014; Nascimento et al., 2016; Motamedi et al., 2018), our results showed that on cut apples, no significant differences were found between control and carnauba wax treated samples.

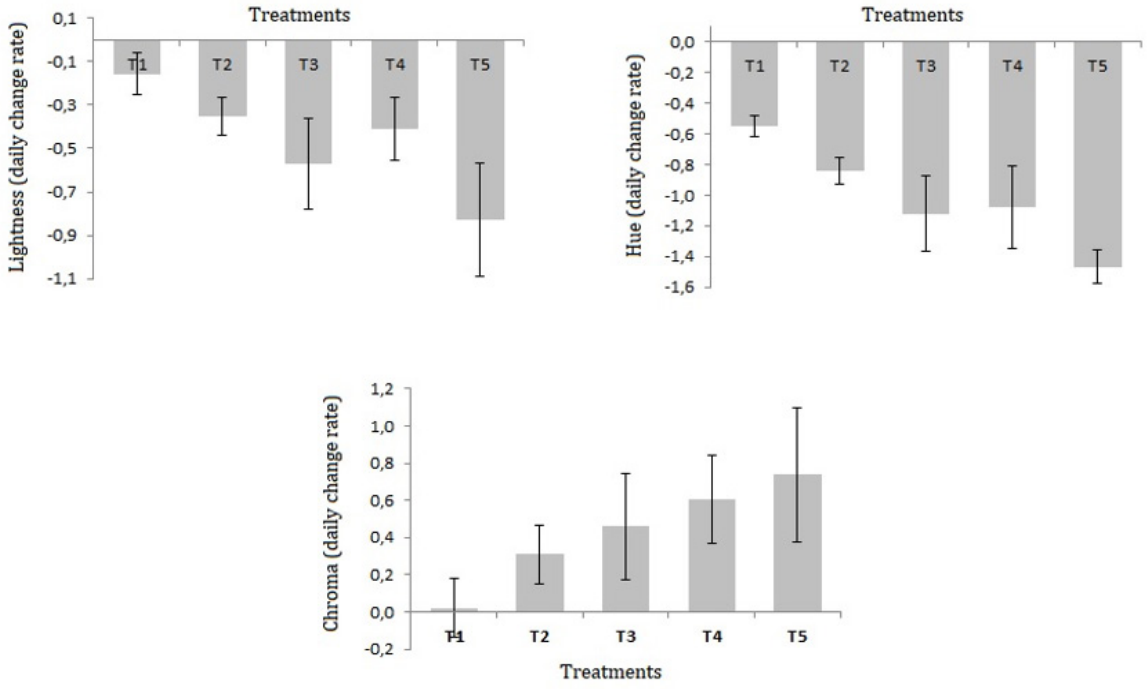

Figure 2. Lightness, Hue and Chroma daily rate, for ten days storage at 5°C (T1=control; T2 = ascorbic acid 1%; T3 = chitosan 1.5%; T4 = 0.5% carnauba wax; T5 = chitosan 1.5% + carnauba wax 0.5%).

Microbiological analyses

For tests with coliforms, *E. coli,* and *Salmonella* no colony growth was observed for all treatments, which may be due to an efficient sanitization by chlorine dioxide and proper handling and storage during the experiment.

For psychotropic bacteria, it was observed a small difference in the initial growth.

Chitosan treatment promoted a delay in initial growth in an average of 2.9±0.27 days to start the colonies appearance. In the other treatments, the initial growth was faster (data not shown).

On the other hand for the growth rate of psychotropic bacteria (Figure 3), it was observed that in the bilayer coating the daily rate is lower compared with the other treatments, and therefore an indicative that the combination of chitosan-carnauba provides an effective inhibition for these bacteria growth. For chitosan individual coating (T3), an intermediate action was observed. Chitosan have been reported as an antimicrobial agent in fresh-cut apples (Pilon et al., 2015), broccolis (Moreira et al., 2011) and mangos (Po-Jung et al., 2007). Chitosan acts through electrostatic interaction between positively charged chitosan molecules and negatively charged microbial cell membranes (Pilon et al., 2015). Such interaction promotes bacterial cell lysis decreasing microorganism population.

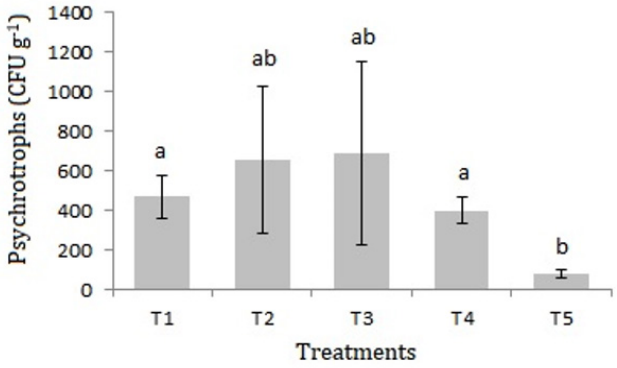

Figure 3. Daily growth rate Psychotropic bacteria. T1=control; T2=ascorbic acid 1%; T3=Chitosan 1.5%; T4=0.5% carnauba wax; T5=Chitosan 1.5% + Carnauba wax 0.5%. Means followed by the same letter on the line do not differ at 5% probability by Duncan test.

For Carnauba individually the antimicrobial action is based on a formation of hydrophobic layers, defaulting microorganism adhesion and subsequent colony formation. Gonçalves et al. (2010) showed that carnauba treatments can be effective in microorganism's inhibition when applied in higher concentrations than the ones used here. In the present study, however, low concentration of carnauba (0.5%) was used since preliminary tests showed that elevated concentrations resulted in pulp darkening. Based on the results it can be suggested that chitosan-carnauba bilayer is effective in reducing bacteria growing and consequently extending of cut apple when storage at low temperatures.

Sensorial analyses

Three parameters were verified for sensorial analysis: coloration, firmness and general appearance (Figures 4, 5 and 6, respectively). The first analyze related to color was conducted five days after coating (Figure 4). T4 (0.5% carnauba wax) and T2 (ascorbic acid 1%) showed the highest number of scores (4-5), and isolated chitosan (T3) and bilayers (T5) the lowest. Concerning firmness (Figure 5), the results were not so expressive in percentage scores. But, for general appearance acceptance, the results were similar as color scores, with a higher acceptance for T4 samples (0.5% carnauba wax) and T2 (ascorbic acid 1%) (Figure 6). Treatments based on isolated chitosan (T3) or bilayers (T5) did not show high percentages in note 4 and 5.

On the other hand, for the second set of analyzes, carried out at ten days after coating, the results showed the color acceptance increases only for the bilayer T5 treatment (Figure 4). An increase in firmness scores (Figure 5) and a general appearance acceptance by consumers (Figure 6), occurs especially in treatments with carnauba wax, individually or in

bilayer form (T4 and T5). These observations can be an indication that carnauba has extensive effect over the days, preserving physical properties.

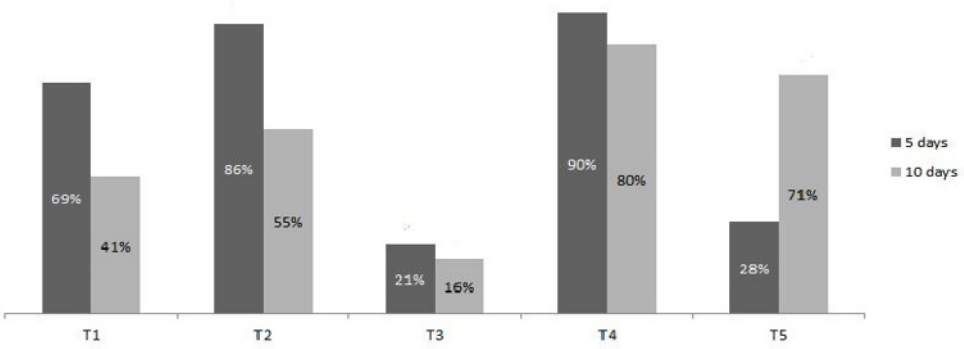

Figure 4. Coloration score (4+5) acceptance consumers. T1 = control; T2 = ascorbic acid 1%; T3 = Chitosan 1.5%; T4 = 0.5% carnauba wax; T5 = Chitosan 1.5% + Carnauba wax 0.5%.

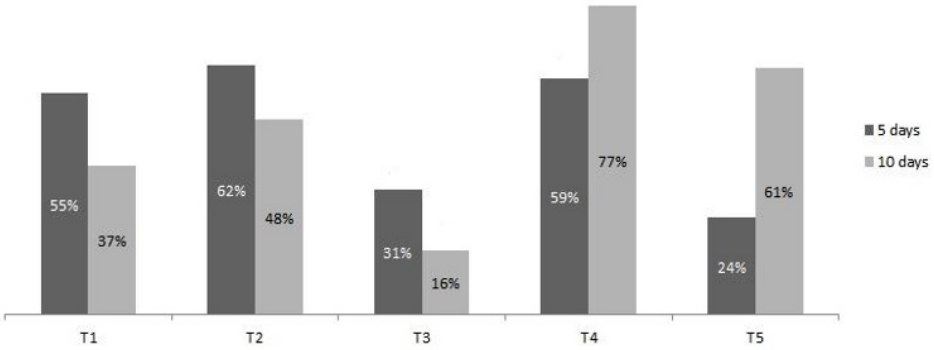

Figure 5. Firmness score (4+5) acceptance consumers. T1 = control; T2 = ascorbic acid 1%; T3 = chitosan 1.5%; T4 = 0.5% carnauba wax; T5 = chitosan 1.5% + carnauba wax 0.5%.

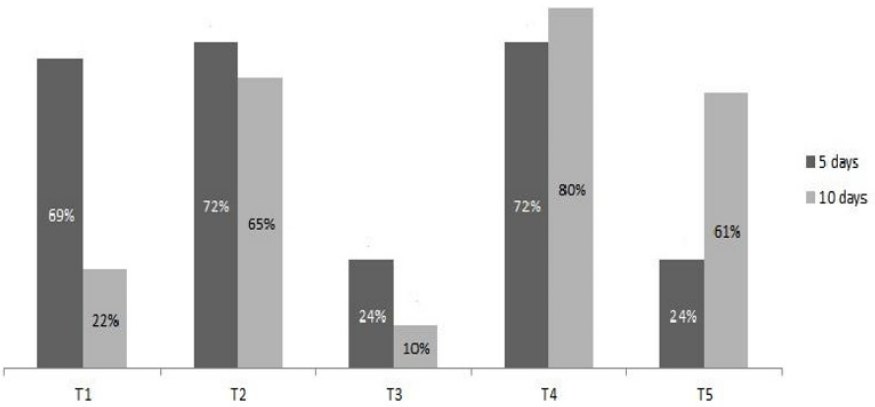

Figure 6. General appearance score (4+5) acceptance consumers.. T1 = control; T2 = ascorbic acid 1%; T3 = chitosan 1.5%; T4 = 0.5% carnauba wax; T5 = chitosan 1.5% + carnauba wax 0.5%.

For some fruits, such as tomato, the general appearance is directly related to firmness, which is a relevant parameter for acceptance (Yang and Chinnan, 1988; Batu, 2004). Apples slices with T4 and T5 treatments had the better general appearance and firmness parameters and consequently the highest acceptance. These results suggest a correlation with shine appearance by carnauba wax coatings.

CONCLUSIONS

Carnauba wax and chitosan were used as coating individually and in bilayer. Chitosan antimicrobial properties were observed in single and bilayer layers; and sensory analyses pointed a greater preference for carnauba treatments, mono or bilayer, which may be related to lightness. Bilayer formation of chitosan and carnauba has a potential to keep quality and extend apple shelf life with consumer preference during storage at low temperatures.

ACKNOWLEDGEMENTS

The authors thank the financial support provided by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Capes – Rede Nanobiotec), *Empresa Brasileira de Pesquisa Agropecuária* (EMBRAPA, Project 02.13.05.003.00.00) and MCTI-SisNano from Brazil.

Literature cited

Acevedo-Fani, A., Soliva-Fortuny, R., and Martin-Belloso, O. (2017). Nanostructured emulsions and nanolaminates for delivery of active ingredients: improving food safety and functionality. Trends Food Sci. Technol. *60*, 12–22 https://doi.org/10.1016/j.tifs.2016.10.027.

Agrianual. (2019). Anuário da Agricultura Brasileira (São Paulo: Agrianual), p.296–301.

Baranyi, J., and Roberts, T.A. (1994). A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. *23* (*3-4*), 277–294 https://doi.org/10.1016/0168-1605(94)90157-0.

Batu, A. (2004). Determination of acceptable firmness and colour values of tomatoes. J. Food Eng. *61* (*3*), 471–475 https://doi.org/10.1016/S0260-8774(03)00141-9.

Chiumarelli, M., and Hubinger, M. D. (2012). Stability, solubility, mechanical and barrier properties of cassava starch–Carnauba wax edible coatings to preserve fresh-cut apples. Food hydrocolloid *28* (*1*), 59–67.

de Freitas, C.A.S., de Sousa, P.H.M., Soares, D.J., da Silva, J.Y.G., Benjamin, S.R., and Guedes, M.I.F. (2019). Carnauba wax uses in food - A review. Food Chem. *291*, 38–48 https://doi.org/10.1016/j.foodchem.2019.03.133. PubMed

Embuscado, M. E., and Huber, K. C. (2009). Edible films and coatings for food applications, 295–314. New York: springer, (9).

Garrido Assis, O.B., and de Britto, D. (2011). Evaluation of the antifungal properties of chitosan coating on cut apples using a non-invasive image analysis technique. Polym. Int. *60* (*6*), 932–936 https://doi.org/10.1002/pi.3039.

Gonçalves, F.P., Martins, M.C., Junior, G.J.S., Lourenço, S.A., and Amorim, L. (2010). Postharvest control of brown rot and Rhizopus rot in plums and nectarines using carnauba wax. Postharvest Biol. Technol. *58* (*3*), 211–217 https://doi.org/10.1016/j.postharvbio.2010.08.004.

Guerreiro, A.C., Gago, C.M., Faleiro, M.L., Miguel, M.G., and Antunes, M.D. (2017). The effect of edible coatings on the nutritional quality of 'Bravo de Esmolfe'fresh-cut apple through shelf-life. LWT – Food Sci Techinol, 75, 210–219.

Hagenmaier, R.D., and Baker, R.A. (1997). Edible Coatings from Morpholine-Free Wax Microemulsions. J. Agr. Food Chem, 45–2, 349–352.

Jo, W.S., Song, H.Y., Song, N.B., Lee, J.H., Min, S.C., and Song, K.B. (2014). Quality and microbial safety of 'Fuji'apples coated with carnauba-shellac wax containing lemongrass oil. LWT – Food Sci Techinol, 55(2), 490–497. https://doi.org/10.1016/j.lwt.2013.10.034

Lin, D., and Zhao, Y. (2007). Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. Compr. Rev. Food Sci. Food Saf. *6* (*3*), 60–75 https://doi.org/10.1111/j.1541-4337.2007.00018.x.

Mantilla, N., Castell-Perez, M.E., Gomes, C., and Moreira, R.G. (2013). Multilayered antimicrobial edible coating and its effect on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*). LWT – Food Sci. Techinol. *51* (*1*), 37–43.

Moreira, M.D.R., Roura, S.I., and Ponce, A. (2011). Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. LWT – Food Sci. Techinol. *44* (*10*), 2335–2341.

Motamedi, E., Nasiri, J., Malidarreh, T.R., Kalantari, S., Naghavi, M.R., and Safari, M. (2018). Performance of carnauba wax-nanoclay emulsion coatings on postharvest quality of 'Valencia'orange fruit. Sci. Hortic. (Amsterdam) *240*, 170–178 https://doi.org/10.1016/j.scienta.2018.06.002.

Nascimento, F.V., Almeida, G.K., Silva, S.J.N., and Bender, R.J. (2016). Coatings based on chitosan and carnauba wax for postharvest use on 'Rocha' pears. Acta Hortic. *1194*, 283–288 https://doi.org/10.17660/ActaHortic.2018.1194.41.

Pilon, L., Spricigo, P.C., Miranda, M., de Moura, M.R., Assis, O.B.G., Mattoso, L.H.C., and Ferreira, M.D. (2015). Chitosan nanoparticle coatings reduce microbial growth on fresh-cut apples while not affecting quality attributes. Int. J. Food Sci. Technol. *50* (*2*), 440–448 https://doi.org/10.1111/ijfs.12616.

Po-Jung, C., Fuu, S., and Feng-Hsu, Y. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. J. Food Eng. *78* (*1*), 225–229 https://doi.org/10.1016/j.jfoodeng.2005.09.022.

Toliba, A.O., Rabie, M.A., and El-Araby, G.M. (2014). Extending the shelf-life of cold stored strawberry by chitosan and carnauba coatings. J. Agric. Res. (Lahore) *41* (*5*), 1067–1076.

Vásconez, M.B., Flores, S.K., Campos, C.A., Alvarado, J., and Gerschenson, L.N. (2009). Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. Food Res. Int. *42* (*7*), 762–769 https://doi.org/10.1016/j.foodres.2009.02.026.

Vieira, J.M., Flores-López, M.L., de Rodríguez, D.J., Sousa, M.C., Vicente, A.A., and Martins, J.T. (2016). Effect of chitosan–Aloe vera coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. Postharvest Biol. Technol. *116*, 88–97 https://doi.org/10.1016/j.postharvbio.2016.01.011.

Yang, C.C., and Chinnan, M.S. (1988). Computer modeling of gas composition and color development of tomatoes stored in polymeric film. J. Food Sci. *53* (*3*), 869–872 https://doi.org/10.1111/j.1365-2621.1988.tb08974.x.