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Chitosan and Carnauba Wax Coatings Are Not Recommended for Yellow Carrots

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Abstract: The objective of this study was to evaluate the use of different concentrations of carnauba wax and chitosan edible coatings for commercial quality preservation of 'Yellow Stone' carrots. Seven treatments were tested: Chitosan at concentrations of 1%, 3%, and 5%; carnauba wax at concentrations of 0.5%, 1%, and 12%, and a control treatment, without coating application. Carrots were stored at 2 °C, 95–100% RH, for 30 days, and were evaluated on the day of application (day 0) and at 7, 15, and 30 days. Indices of brown stains, coloring, and light microscopy analysis were developed. The use of edible coatings for yellow carrots was not viable, regardless of the treatment used, and carnauba waxes caused more severe brown stains. Higher concentrations of carnauba wax caused damage of the carrot periderm, generating, in addition to the stains, deep depressions and superficial viscosity. Only the control treatment showed no degradation in appearance. Treatments with the highest index scores presented lower luminosity, lower b color values, and higher a color values, which showed that the brown stains impacted carrot appearance and, therefore, their visual quality. The results showed that coatings based on chitosan and carnauba wax are not recommended for yellow carrots, since they negatively affected appearance of the product, leaving them unmarketable.

Keywords: *Daucus carota* L.; wilting; damages; microscopy; appearance

1. Introduction

Postharvest losses of horticultural products restrict the availability of food in the world. With carrots, losses happen due to physical or esthetic flaws as a result of mechanical injuries, pathogen attack, shape defects, and withering [1,2]. Other studies led by our research group indicated that yellow carrots lose about 15% of fresh weight in only seven days when stored under ambient conditions (25 °C and 70% RH) (data not published).

Yellow carrots were recently embraced by Brazilian growers, appealing as a distinctive gourmet product, but one that does not have constant availability in the market. Better quality roots are obtained during a winter harvest and must be stored to maintain their availability for the longest time possible. However, this standard will only be achieved by adopting good postharvest practices, associated with the use of technologies that help to minimize losses and maintain the quality of the products for a longer period.

Edible coatings are considered the packaging of the future and may help reduce losses caused by withering (i.e., water loss. These coatings are thin layers of edible biopolymers made of proteins, polysaccharides, or lipids, which are applied directly onto a product surface, adhering to it as part of the final product. These materials create a barrier against physical injuries, microbiological contamination, loss or gain of moisture, and nutrient oxidation. Therefore, they help prevent product deterioration,

extending storage life, sensorial quality, and safety. The difference between these coatings and plastic packaging is the fact that the former is edible and biodegradable, and may substitute partially or totally for the latter [3,4].

Chitosan is the basis of edible coatings from non-starch polysaccharides. It is made by alkaline deacetylation of chitin, which is a polysaccharide present in the exoskeleton of crustaceans. Besides the usual properties of coatings, the film generated by chitosan provides a good barrier to oxygen, although it is not an efficient barrier against carbon dioxide. It also contributes to the control of enzymatic browning and microbial activity, as well as the capacity of absorbing ions of heavy metals. These properties play a role in minimizing the oxidation process catalyzed by free metals and delay changes in the content of anthocyanins, flavonoids, and total phenolics [3,5,6].

Among lipid coatings, natural waxes, such as carnauba wax, stand out. They are chemically classified as esters of long chain aliphatic acids. As a non-polar compound, they are an efficient barrier to the exchange of water vapor. Carnauba wax has better emulsifying, lubricity, plasticising, and adhering properties than other waxes, besides a greater permeability to O_2. It also improves food appearance by providing gloss and improving superficial texture [7].

There are few studies that show the effects of edible coatings on carrot quality. Thus, our objective was to evaluate the use of different concentrations of two types of edible coatings, one of carnauba wax and the other of chitosan, on the conservation of the commercial quality of yellow carrots.

2. Materials and Methods

2.1. Raw Material and Treatments

'Yellow Stone' carrots were obtained from a commercial farmer (Araçoiaba da Serra, SP, Brazil, latitude, 23°30′19″ S, and longitude, 47°36′51″ O). Carrots were divided among seven treatments: (1) Control treatment (without coating application); (2) chitosan at a concentration of 1%; (3) chitosan at 3%; (4) chitosan at 5%; (5) carnauba wax at 0.5%; (6) carnauba wax at 1%; and (7) carnauba wax at 12%.

Chitosan (Nutrifarm do Brazil, São Paulo, Brazil, low molecular weight) was dissolved in a solution of 8% acetic acid and 1% glycerol. For applying coating, carrots were immersed in the solutions for 5 min and then kept on absorbent paper until the coating was fully dried. The carnauba wax with colophony (Aruá Comércio e Serviço LTDA., São Paulo, Brazil, BR12) was obtained at a concentration of 12% and diluted in distilled water for lower concentrations. Colophony is a wood rosin that acts as an emulsifier in carnauba wax. The wax application was made by using a cotton wool soaked in the solution. Then, carrots were laid on absorbent paper until the coating was fully dried. Finally, carrots were transferred to plastic containers (20 kg capacity) and stored at 2 °C, 95–100% RH for 30 days.

Analyses were performed on the day of coating applications (day 0) and after 7, 15, and 30 days. The study used a randomized experimental design with a factorial structure of 7 × 4 (treatments × days of analysis), with five repetitions of seven carrots (approximately 500 g) each.

2.2. Analisys

Brown stain index (BSI) was always performed by the same analyst by rating carrots based on visual appearance. Scores ranged from 1 to 3, being 1 = roots free of stains; 2 = roots with light or few stains; 3 = roots with a large amount and/or darker stains (Figure S1). The number of carrots that received each score was converted into a percentage and the result was expressed as % of roots. The shelf life limit was determined when there were no more carrots rated as 1. They were discarded at that time.

Color was evaluated by using a MINOLTA CHROMA METER CR-400 and the luminosity L*, a* and b* values were determined. Value a* is a red/green coordinate (+a indicates red and −a indicates green) and value b* is a yellow/blue coordinate (+b indicates yellow and −b indicates blue) [8]. Three

measurements were taken on the external surface of each of the seven carrots per repetition, one in the upper area, one in the middle area, and one in the lower area.

To determine the effects of these edible coatings on the carrots, periderm anatomical analyses were performed after 15 days of storage. An optical microscopy analysis was performed in both healthy and damaged carrots on cross-sectional cuts made with a sliding microtome Leica SN 2000 R (Leica Biosystems, Heidelberg, Germany), clarified in a bleach solution 10%, and washed with distilled water. Histochemical tests were conducted with Sudan IV for lipid substances [9] and double color with iodine green (greenish-blue color for lignin) and Congo red (red color for cellulose) [10]. Images were captured by a trinocular Leica DM LB microscope (Leica Microsystems, Wetzlar, Germany) connected to a Leica DC 300 F video camera. External images of the carrots were captured by aM205C (lens) microscope (Leica Microsystems, Wetzlar, Germany) connected to a Leica DFC 295 video camera.

The BSI and color results were submitted to a variance analysis (ANOVA) and the averages were compared by the Tukey test ($P \leq 0.05$), using the statistical software SISVAR, version 5.6 [11].

3. Results and Discussion

3.1. Visual Quality

To determine the effect of edible coatings on the quality of yellow carrots, changes in visual characteristics were observed. General appearance was taken as a marketing criteria. Overall, the use of edible coatings on yellow carrots was not viable, irrespective of treatment. The 12% carnauba wax 12% brown stains on the periderm right after the application and the intensity of these stains increased during storage (Figure 1). Carnauba wax at the lower concentrations (0.5% and 1%) and all chitosan coatings did not cause stains on the day of application. Except for the control treatment, all treated carrots presented periderm stains on the seventh day of storage. On this day, all the carnauba treatments and 3% chitosan 3% presented more severe stains, receiving a score of 3. Treatments with 1% and 5% chitosan exhibited light or few stains after seven days of storage. On the 15th day of storage, carrots coated with carnauba wax were discarded due to the severity of the stains. For 1%, 3%, and 5% chitosan-coated carrots, only 27%, 10%, and 13% did not have stains, respectively. The control treatment exhibited 7% of the carrots with light stains. On the 30th storage day, despite the concentration, 100% of the carrots coated with chitosan were stained and thus not marketable.

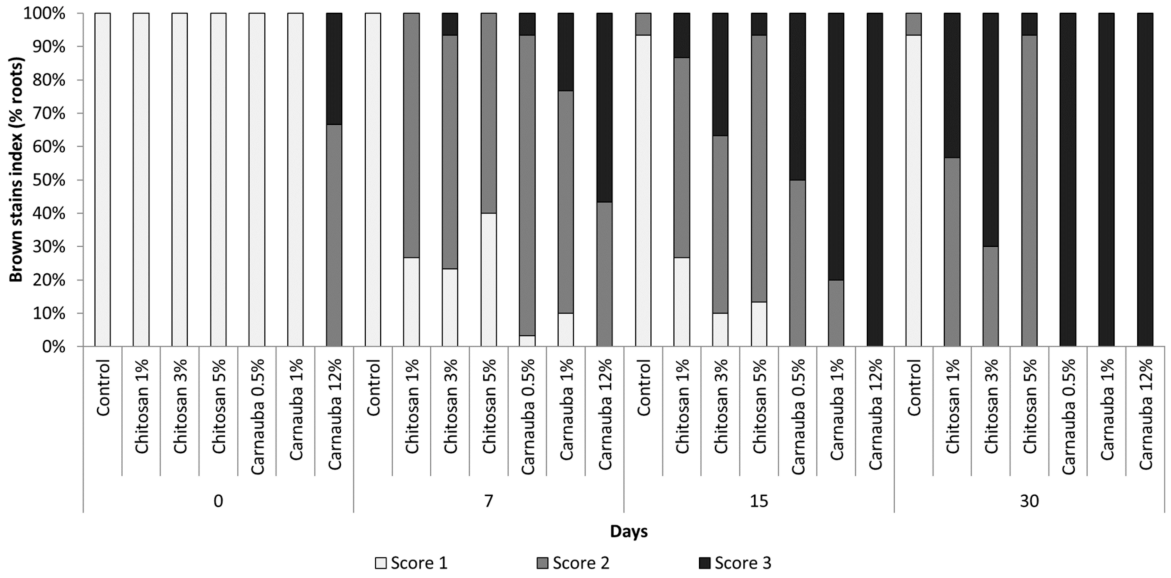

Figure 1. Brown stain index of yellow carrots treated with different edible coatings and stored at 2 °C 95–100% RH. Score: 1 = roots without stains; 2 = roots with light or few stains; 3 = roots with a large amount of stains or with darker stains.

The color of yellow carrots was affected by the application of coatings, confirming the data obtained from the visual rating. The luminosity decreased during cold storage for all treatments (Figure 2A). Except for 1% chitosan, all treatments presented lower luminosity values than the control samples. The decline in luminosity was proportional to the increase of the coating concentration. The reduction on the L* values indicated the darkening of the surface caused by the brown stains.

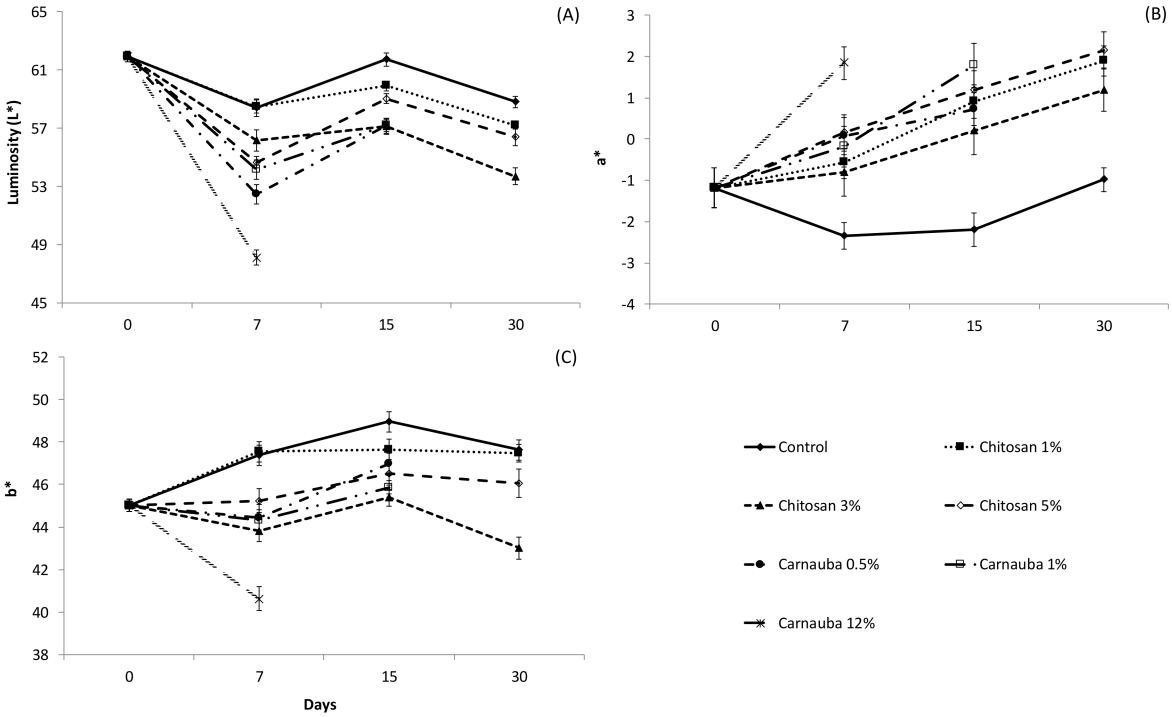

Figure 2. Luminosity (**A**) and values a* (**B**), and b* (**C**) of yellow carrots treated with different edible coatings and stored at 2 °C 95–100% RH. Vertical bars represent the standard error of the mean (n = 5).

The control treatment did not show significant variation for the a* values during cold storage, always exhibiting the lowest values among the different treatments (Figure 2B). All of the other samples presented a* values that increased during storage, which indicated a change in the characteristic yellow color of this variety. The higher the a* value, the closer the sample is to a reddish hue. Brown stains in these treatments left a reddish tint to the carrots, which is directly related to the increase in the values of a*.

Control, 1% chitosan 1%, and 5% carnauba treatments showed an increase in the values of b* during storage. Chitosan at 1% interfered the least on the color of the yellow carrots, not differing from the control (Figure 2C). Carrots coated with 3% chitosan and 12% carnauba showed a reduction in the b* values from the beginning to the end of the storage period. This reduction showed a loss in the yellow color saturation, especially for the carrots coated with 12% carnauba, which also exhibited the highest degree of brown stains. Carrots coated with 5% chitosan and 1% carnauba 1% did not show any variation; however, their b* values were lower than the control.

3.2. Damage Evaluation with Microscope and Magnifier

Due to the observation of visual quality losses caused by the coatings, microscopy and magnifying glass techniques were used to investigate the extent of damage.

After 15 days of storage, damage affected the carrot periderm. Carnauba at 12% caused not only stains, but also depressions and superficial viscosity (Figure 3F). Carrots with chitosan presented lighter stains than the carnauba after 15 days of storage. However, at the end of the storage period, the stains were darker, similar to the ones caused by the carnauba.

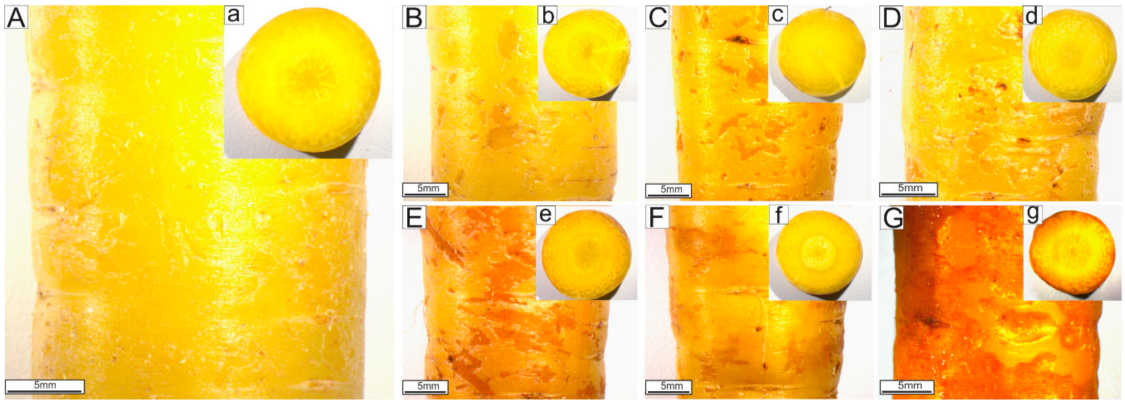

Figure 3. Details of yellow carrots 15 days after the application of different edible coatings and stored at 2 °C 95–100% RH. (**A**,**a**) Control; (**B**,**b**) chitosan 1%; (**C**,**c**) chitosan 3%; (**D**,**d**) chitosan 5%; (**E**,**e**) carnauba wax 0.5%; (**F**,**f**) carnauba wax 1%; (**G**,**g**) carnauba wax 12%.

Carnauba wax at 6% (Figure 4A) was also tested (no data presented) and, like 12% carnauba (Figure 4B), it caused brown stains immediately after the coating application. The carrot surface has a periderm full of imperfections, creating a debarking aspect. Apparently, stains were concentrated on these imperfections along the carrot, suggesting that these structures were more sensitive to carnauba than the ones covered by the periderm. As the days advanced, stains spread and damaged the periderm cells, causing bigger injuries. Carnauba at 12% caused browning of the inner part on a significant area of the secondary phloem. The intensity of the injury also induced the occurrence of viscosity over the stains (Figures 3F and 4B) and depressions (Figure 4). Depressions were generated by the compaction of periderm and parenchyma cells (Figure 4C,E). In some cases, there was periderm rupture (Figure 4D,F) and deposition of lignin on the depressed region (Figure 4F). It was possible to observe that the damaged regions frequently occurred close to the secretory canals, which are located longitudinally in the root. This intensifies the depression and brings about the rupture and exposition of these canals (Figure 4F).

Brown stains probably resulted from the oxidation of phenolic compounds and the subsequent formation of darker compounds. The interaction between the coating and the cells seems to have degraded cell structures, and thus this decompartmentalization provided the contact between phenolic compounds and oxidative enzymes. The *o*-quinones, which are products of this reaction, have an undesirable brown color that stands out on the yellow carrot surface. It depreciates its appearance and commercial quality.

The emergence of stains on the treatments with carnauba wax may be related to the basic pH of the product (around 9.0) that remains unaltered after its dilution. Solutions that are very basic may cause injuries on the cell walls, such as burns. Caron et al. [12] also reported the emergence of stains on 'Brasília' carrots. These stains were caused by the application of carnauba coatings undiluted or in concentrations of 1:3 and 1:6, depending on the brand of coating used.

Based on these results, chitosan and carnauba wax coatings at the tested concentrations are not recommended for yellow carrots, as they damaged the carrots appearance, thus making them unmarketable.

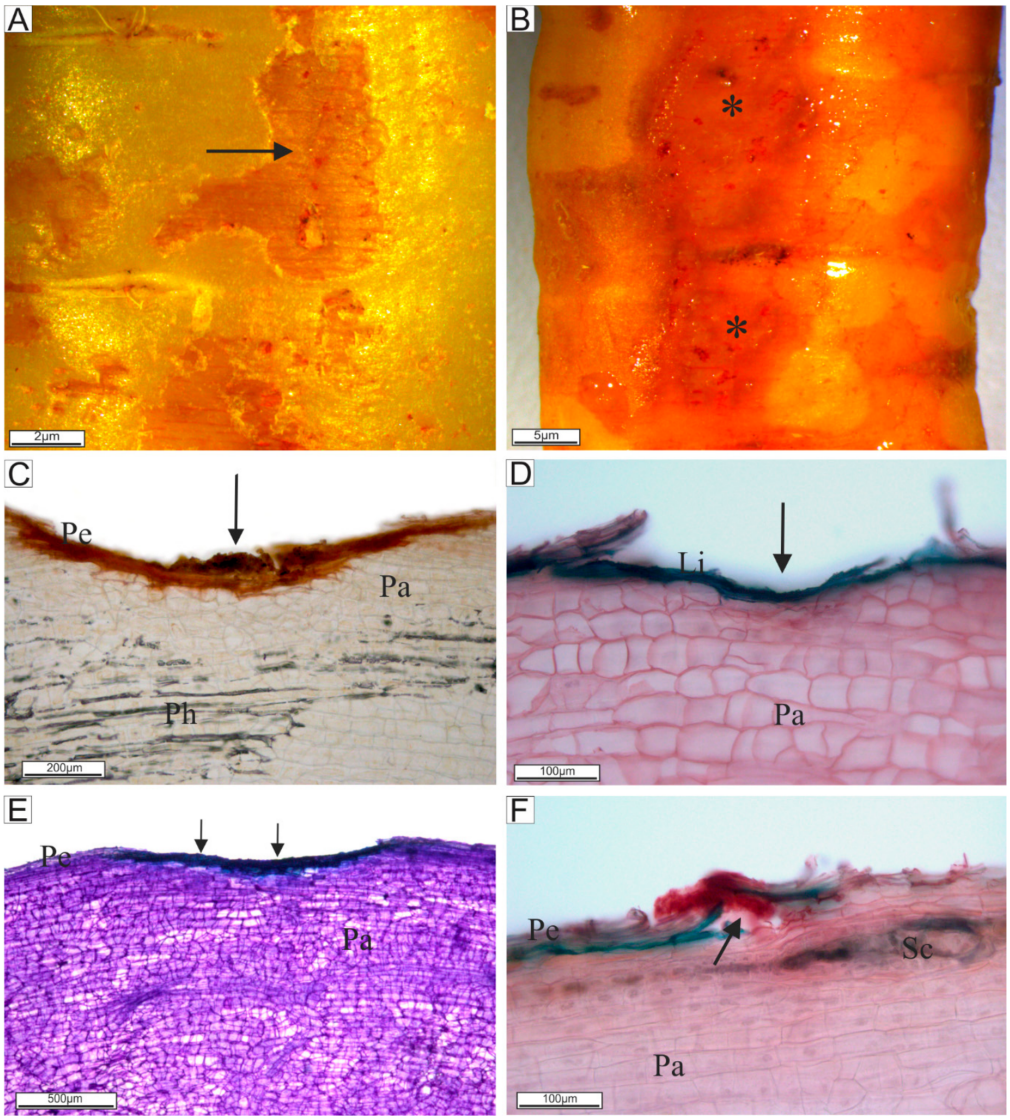

Figure 4. Damage to yellow carrots promoted by the carnauba wax: (**A**) Carnauba wax 6%; (**B**) carnauba wax 12%; (**C**) carnauba wax 6%; and (**D**) carnauba wax 12%. Photomicrographs of cross sections of superficial region of carrots: (**C**) Depression and compaction of the periderm. (**D**) Rupture and compaction of periderm cells evidenced by lignin deposition. (**E**) Compression of the periderm layers. (**F**) Periderm rupture near the secretory canal. Sc = Secretory canal; Ph = Phloem; Li = Lignin; Pa = Parenchyma; Pe = Periderm (arrows = depressions; * regions with viscous appearance).

Supplementary Materials: The following are available online at http://www.mdpi.com/2311-7524/4/4/31/s1, Figure S1: Score of Brown Stains Index in yellow carrots. 1 = roots without stains; 2 = roots with light stains or few stains; 3 = roots with large amount of stains or with darker stains, Table S1: Color of yellow carrots treated with different edible coatings and stored at 2 °C 95–100% RH.

Author Contributions: N.D.B., R.A.K. and M.H.F.S. conceived and designed the experiments; N.D.B., M.A.T., A.P.P. and B.T.N. performed the experiments, analyzed the data and wrote the paper. R.A.K. and M.H.F.S. edited and revised the paper.

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Conflicts of Interest: The authors declare no conflict of interest.

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