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ORIGINAL ARTICLE

Conservation of minimally processed pinhão using chitosan and gelatin coatings

Conservação de pinhão minimamente processado pelo uso de revestimentos de quitosana e gelatina

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Abstract

The minimal processing of pinhão causes an increase in mass loss, physiological deterioration and the growth of microorganisms. Thus, this study aimed to evaluate the conservation of minimally processed pinhão using edible chitosan and gelatin coatings. The pinhões were minimally processed and coated with chitosan, gelatin and chitosan/gelatin using the Layer-by-Layer (LbL) technique. They were then dried under forced ventilation, packaged in a polyethylene terephthalate package and stored at 4 °C for 10 days. The analyses performed were weight loss, pH, reducing sugars, vitamin C, color, microbiological analysis and sensory analysis. Benefits were observed with the use of chitosan and gelatin coatings, especially when applied using the LbL technique. The best combination of results was obtained with the application of the chitosan/gelatin coating, mainly reduction of weight loss and inhibition of growth of fungi and aerobic psychrotrophic bacteria. The coating did not retard the maturation process, thus, higher vitamin C contents were obtained. The coatings did not influence the taste and aroma of minimally processed pinhão. Minimal processing can encourage the consumption of seeds, besides this, the conservation using edible coating based on chitosan/gelatin, applied with the LbL technique associated with refrigeration, extended their shelf life.

Keywords: *Araucaria angustifolia* (Bertoloni) Otto Kuntze; Seed; Edible coating; Layer-by-layer; Weight loss; Fungi; Psychrotrophic.

Resumo

O processamento mínimo do pinhão ocasiona aumento na perda de massa, deterioração fisiológica e crescimento de microrganismos. Assim, objetivou-se com este estudo avaliar a conservação de pinhão minimamente processado usando revestimentos de quitosana e gelatina. Os pinhões foram minimamente processados e revestidos com quitosana, gelatina e quitosana/gelatina, pela técnica camada sobre camada. Em seguida, foram secos sob ventilação forçada, acondicionados em embalagem de polietileno tereftalato e estocados a 4 °C por 10 dias. Foram realizadas análises de perda de massa, pH, açúcares redutores, vitamina C e cor, além de análises microbiológicas e análise sensorial. Benefícios foram observados com o uso dos revestimentos de quitosana e gelatina, especialmente quando aplicados pela técnica camada sobre camada. A melhor combinação de resultados foi obtida com a aplicação do revestimento quitosana/gelatina, que possibilitou a redução da perda de massa e do crescimento de fungos e bactérias psicrotróficas aeróbias. O revestimento não retardou o processo de maturação e, consequentemente, maiores teores de vitamina C foram obtidos. Os revestimentos não influenciaram o sabor e o aroma dos pinhões minimamente processados. O processamento mínimo pode incentivar o consumo da semente e, além disso, a conservação, utilizando revestimento comestível à base de quitosana/gelatina pela técnica *layer-by-layer*, associado à refrigeração, ampliou a vida útil das sementes.

Palavras-chave: *Araucaria angustifolia* (Bertoloni) Otto Kuntze; Semente; Revestimento comestível; Camada sobre camada; Perda de massa; Fungos; Psicrotróficos.

1 Introduction

The Paraná pine [*Araucaria angustifolia* (Bertoloni) Otto Kuntze] is a native species from Brazil, mainly distributed by the states of Paraná, Santa Catarina and Rio Grande do Sul (Carvalho, 1994). Pinhão is the edible seed of this *Araucaria* and has important nutritional value, representing a rich source of carbohydrates with low glycemic index and fiber, and significant amounts of vitamin C (Universidade Estadual de Campinas, 2011).

For consumption, the pinhão needs to be subjected to cooking and removal of its thick bark. Thus, selling the seed in the minimally processed form facilitates consumption while reducing cooking time. However, according to the results of Moreira et al. (2020), the minimal processing of pinhão could cause increased weight loss, physiological deterioration and the growth of microorganisms. One way to reduce such changes is the application of edible coatings, because they act mainly as a barrier to gases and water vapor, thus modifying the internal atmosphere, reducing changes and leading to increase in the shelf life of vegetables (Maia et al., 2000).

Often the use of a single polymer does not provide adequate coating, so more than one polymer can be used in the Layer-by-Layer (LbL) technique. This technique deposits layer over layer of coatings containing oppositely charged polyelectrolytes, with the aim of improving physical properties from the formation of new chemical bonds (Chen et al., 2008; Denavi et al., 2009).

The LbL technique has already been applied to melon, using alginate and chitosan (Poverenov et al., 2014), and chitosan and pectin (Moreira et al., 2014); to mandarin, using chitosan and carboxymethylcellulose (Arnon et al., 2015); to watermelon, using alginate and pectin (Sipahi et al., 2013); to pineapple, using alginate and pectin (Mantilla et al., 2013), chitosan and pullulan; chitosan and flaxseed mucilage; chitosan and aloe vera; and chitosan mucilage and nopal cactus mucilage (Treviño-Garza et al., 2017). The results depended on the raw material and the type of coating. Among the materials that can be used for vegetable coating with this technique are chitosan and gelatin.

Chitosan is a biopolymer obtained from the deacetylation of chitin from crustacean shells such as shrimp shells, insect shells and fungi (Moura et al., 2011). It is a cationic polysaccharide below pH 6.0 (Roy et al., 2017).

Gelatin is a biopolymer produced from partial collagen hydrolysis. It has a negative charge above isoelectric point pH 4.8-5.0. Thus, gelatin and chitosan can form complexes by electrostatic interactions, in addition to hydrogen bonds, allowing the use of the LbL technique (Qiao et al., 2017).

The study aimed to evaluate the conservation of minimally processed pinhão using edible chitosan and gelatin coatings.

2 Material and methods

2.1 Material

Pinhão samples [*Araucaria angustifolia* (Bertoloni) Otto Kuntze] were obtained from a producer in Vacaria (Latitude: 28°30'39" S, Longitude: 50° 55' 47" W), in the state of Rio Grande do Sul (RS), Brazil. The seeds were collected, packed in a burlap bag and transported to the city of Pelotas (RS), where they were stored at 18 °C for two days until processing.

2.2 Methods

The pinhões were selected, and those one that showed deterioration or apparent fungal growth were discarded. They were then washed with water, sanitized in a sodium hypochlorite solution (200 ppm) at pH 6.5 to 7.0 for 15 min, rinsed with water and peeled manually. After peeling, they were again sanitized, rinsed and centrifuged for 30s.

The chitosan dispersion (Polymar, Fortaleza, Brazil) was prepared at a concentration of 1.5% (w/v) in a 1.5% (w/v) acetic acid solution and homogenized on a magnetic stirrer at room temperature for 2 h. Afterwards, it was heated at 60 °C for 20 min. The dispersion was used 24 h after preparation. The final pH of the solution was 3.12.

The gelatin dispersion (Royal, Pedreira, Brazil) was prepared in distilled water at a 5% (w/v) concentration. This dispersion was homogenized on a magnetic stirrer at 60 °C for 30 min. The final pH of the solution was 5.76. After dissolution, both dispersions were added with 1% (w/v) glycerol.

The following treatments were evaluated: Treatment A - control (deionized water); Treatment B - chitosan (1.5% w/v); Treatment C - gelatin (5% w/v); Treatment D - chitosan (1.5% w/v) under gelatin (5% w/v).

The pinhões submitted to treatments A, B and C were immersed for 30 s in the respective dispersions and dried under forced ventilation at room temperature (15 °C).

Treatment D received the LbL technique, in which the pinhões were first immersed in the chitosan dispersion for 30 s and subjected to drying under the same conditions described above, and after the pinhões were immersed in the gelatin dispersion and subjected to drying.

Finally, the samples were packed in PET (polyethylene terephthalate) thermoformed packaging with lid, standardized at 20 pinhões per package and stored at 4 °C for 10 days. Analyses were performed at least in triplicate, after 0, 3, 6 and 10 days of storage.

2.2.1 Physical and chemical analysis

Weight loss was obtained by calculating the difference between the initial mass of the pinhão and the mass obtained at the end of each storage time (Akhtar et al., 2010), and expressed as percentage of weight loss (Equation 1).

$$Weight\,loss(\%) = \left[\frac{(initial\,mass - final\,mass)}{(initial\,mass)}\right] \times 100 \quad (1)$$

The pH was determined in a suspension prepared with 10 g of peeled pinhão, ground and homogenized with 100 mL of distilled water, with the aid of a potentiometer (Kasvi, Curitiba, Brazil) (Instituto Adolfo Lutz, 2008).

An aqueous extract was prepared from peeled and ground pinhões (2.5 g) in 50 mL of water, by constant stirring for 2 h, and filtered through a qualitative paper. Reducing sugars were determined using the methodology described by Vasconcelos et al. (2013). Aliquots of 1.0 mL of aqueous extract and 1.0 mL of 3,5-dinitrosalicylic acid reagent were pipetted, transferred to a 10 mL volumetric flask and vortexed for 1 min. Subsequently, the sample was placed in a 100 °C water bath for 5 min and later cooled in a cold water bath. The volume of the flask was filled with distilled water. The absorbance of the resulting solution was measured by a spectrophotometer (AAKER, Porto Alegre, Brazil) at 540 nm. The results were quantified using a glucose calibration curve at concentrations of 0 to 3 $mg.mL^{-1}$ (517.88x + 45.851, $R^2 = 0.9907$) and expressed as g per 100 g of pinhões.

For the determination of vitamin C, the pinhões were peeled and ground, and 20 g were transferred to an Erlenmeyer flask, followed by 50 mL of water, 10 mL of 20% (v/v) sulfuric acid solution, 1 mL of 10% (v/v) potassium iodide and 1 mL of 1% (w/v) starch solution. The sample was titrated with 0.02 $mol.L^{-1}$ potassium iodate solution to a pink color, and the result was expressed as mg 100 g^{-1} (Instituto Adolfo Lutz, 2008).

The color was determined using a colorimeter (Minolta, CR 400, Tokyo, Japan). In the CIELAB, the standard was denoted as L* a* b*, where the L* coordinate expresses the degree of lightness of the measured color (L* = 100 = white; L* = 0 = black), the a* coordinate expresses the degree of variation between red (+60) and green (–60) and the b* coordinate expresses the degree of variation between blue (–60) and yellow (+60).

2.2.2 Microbiological analysis

Microbiological analysis was performed according to the procedures proposed by Downes & Ito (2001). Serial dilutions were made in 0.1% buffered peptone water up to 10^{-4} dilution, and the analysis was performed in duplicate.

Quantification of aerobic psychrotrophic microorganisms was performed by plating dilutions on Plate Count Agar (PCA), and the plates were incubated at 7 °C for 10 days. The microbial count was expressed in CFU g^{-1}.

The Potato Dextrose Agar plating method was used for fungal enumeration (molds and yeasts), and the plates were incubated at 25 °C. Counts were obtained at 3 and 5 days of incubation. The result was expressed in CFU g^{-1}.

2.2.3 Sensory analysis

The samples of the minimally processed pinhões were cooked for 20 min in a pressure cooker and sensorially evaluated according to the Triangular Test, in three repetitions. In this test, the evaluator received simultaneously three samples, being two equal and one different, with the purpose of distinguishing the taste and aroma of the different sample (Instituto Adolfo Lutz, 2008). Twenty appraisers familiar with the technique participated in the analysis. Individual booths were used, in which the samples were offered in non-disposable white porcelain containers and coded with three digits. The test was approved by the Ethics Committee of the School of Medicine of the Federal University of Pelotas, under number CAAE 12348019.1.0000.5317. Participants provided written consent.

2.2.4 Statistical analysis

The results were subjected to Analysis of Variance (ANOVA), and means between treatments were compared using Tukey's test with a significance level of 5%, using the Statistix 10 software (USA) (Analytical Software, 2019). For the evaluation of storage time, the confidence interval was set at 95%. For the sensory analysis, the hit percentages were analyzed according to the significance level table (5%) in the triangular test (Instituto Adolfo Lutz, 2008).

3 Results and discussion

3.1 Physical and chemical analysis

By analyzing the data of weight loss of minimally processed pinhões in relation to time, it could be observed that there was a significant increase ($p \leq 0.05$) of the values during storage, in all treatments, as shown in Figure 1A. At the end of storage, it was observed that minimally processed gelatin-coated pinhões showed significantly lower mass loss ($p \leq 0.05$), with a reduction of approximately 23% when compared to the control and chitosan treatments (Supporting information A).

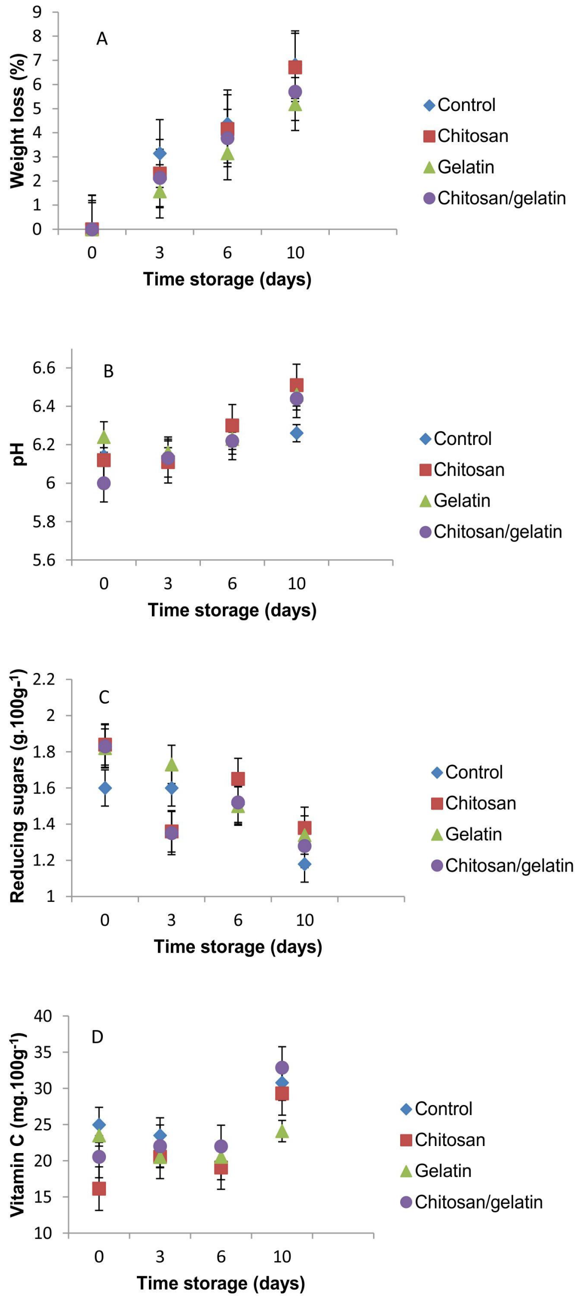

Figure 1. Weight loss (A), pH (B), reducing sugars (C) and vitamin C (D) in minimally processed pinhões coated with chitosan, gelatin and chitosan/gelatin using the Layer-by-Layer (LbL) technique, stored under refrigeration at 4 °C for 10 days. Vertical bars represent the 95% of confidence interval.

Regarding the chitosan/gelatin coating using the LbL technique, it seems that the presence of chitosan reduced the coating efficiency, obtaining a 16.5% of reduction in weight loss. Possibly, the presence of chitosan has reduced the adherence of gelatin to the pinhão. According to Lin & Zhao (2007), the viscosity, density and surface tension of the coating were factors that influence adhesion.

Weight loss in pinhões results mainly from increased water loss, as well as increased consumption of respiratory substrates, due to energy expenditure and disorganization associated with dehydration and tissue senescence (Amarante et al., 2007).

Thus, by using edible coatings, it is desired that they restrict the diffusion of water vapor and create a saturated atmosphere between the film and the surface of the vegetable (Oliveira & Santos, 2015).

Moreira et al. (2020), when evaluating the conservation of minimally processed pinhões using coatings based on chitosan and xanthan gum/clove essential oil, applied alone, did not observe a reduction in weight loss in relation to the control, unlike results obtained in this study.

On the other hand, Arnon et al. (2015) obtained greater reduction of tangerine weight loss when using carboxymethylcellulose/chitosan coating with the LbL technique, in relation to the use of polymers alone. Other authors have also demonstrated the efficiency of the technique in reducing the weight loss of minimally processed vegetables (Sipahi et al., 2013; Treviño-Garza et al., 2017). However, Poverenov et al. (2014) observed a greater reduction of mass loss in peppers when using chitosan and gelatin alone than when they were applied together.

A significant increase in the pH values of minimally processed pinhões during storage can be observed in all treatments ($p \leq 0.05$) (Figure 1B). At the end of storage, it was observed that the control sample had lower pH values (6.26) ($p \leq 0.05$), with significant distinction only in relation to the minimally processed pinhões coated with chitosan (6.51) (Supporting information B). Thus, there is no relation with the presence of acetic acid in the preparation of chitosan dispersion. High pH values may be associated with the use of excess organic acids as a respiratory substrate (Araújo & Shirai, 2016).

According to Chitarra & Chitarra (2005), the acid content in vegetables decreases with maturation, consequently increasing the pH as a function of respiration and the conversion of organic acids into sugars. The increase in acidity during ripening can be attributed to the formation of organic acids from cell wall degradation (Pereira et al., 2005).

Similar behavior was obtained by Moreira et al. (2020), in which a reduction in the acidity of the minimally processed pinhões coated with chitosan and xanthan/clove essential oil was observed during refrigerated storage. On the other hand, the efficiency of coatings applied to vegetables with the LbL technique in maintaining pH seems to be dependent on factors such as raw material, polymers and their concentration (Mantilla et al., 2013; Treviño-Garza et al., 2017).

Significant reduction ($p \leq 0.05$) in the reducing sugar content of the minimally processed pinhões was observed in all treatments during storage, as shown in Figure 1C. On the tenth day of storage, the control sample presented significantly the lowest content of reducing sugars ($p \leq 0.05$) (1.18 g.100 g^{-1} pinhão) in relation to the pinhões with added coating (Supporting information C). Among the coatings, gelatin and chitosan provided the release of higher reducing sugar content in the pinhão ($p \leq 0.05$), in relation to the added sample of these coatings using the LbL technique.

According to Cordenunsi et al. (2004), starch is the main carbohydrate present in pinhões (36%). Its degradation can occur by enzymatic hydrolysis and phosphorolysis, increasing the sugar content (Bewley & Black, 1994).

Moreira et al. (2020) observed an increase in reducing sugars in minimally processed pinhões, which differs from the findings in this study. Possibly, the reduction in reducing sugar content observed in minimally processed pinhões is related to the increase in vitamin C content.

There was a significant increase ($p \leq 0.05$) in the vitamin C content of minimally processed pinhões submitted to control treatment and those coated with chitosan and chitosan/gelatin during the 10 days of refrigerated storage. With the use of gelatin-based coating, in general, there was no significant variation during storage ($p \geq 0.05$), as shown in Figure 1D. Thus, at the end of storage, vitamin C values in gelatin-coated pinhões (24.1 mg.100 g^{-1}) were significantly lower ($p \leq 0.05$) compared to control pinhões (30.77 mg.100 g^{-1}) and those coated with chitosan/gelatin (32.85 mg.100 g^{-1}) (Supporting information D).

According to Coultate (2004), vitamin C is formed from a simple sugar, such as glucose, during the vegetable's maturation process. Thus, it is suggested that the observed increase in vitamin C content in the control, chitosan and chitosan/gelatin treatments is related to the conversion of sugars to ascorbic acid.

Similar values in the vitamin C content of minimally processed pinhões were obtained by Moreira et al. (2020). However, different behaviors were observed, depending on the coating.

Mantilla et al. (2013) and Treviño-Garza et al. (2017), using the LbL technique for coating minimally processed pineapple, observed a reduction in vitamin C content in all treatments, unlike the behavior observed in this work. Regarding the influence of the coating, Poverenov et al. (2014) did not observe significant distinction in vitamin C content when using chitosan and gelatin alone, in relation to their application together.

Regarding the L* coordinate, the values fluctuated during storage, with a tendency to maintenance when comparing the first and last storage days ($p \geq 0.05$) (Figure 2A). Chitosan-coated pinhões (62.15) showed significantly lower L* values ($p \leq 0.05$) than control (67.31) and those treated with chitosan/gelatin (66.84) (Supporting information E).

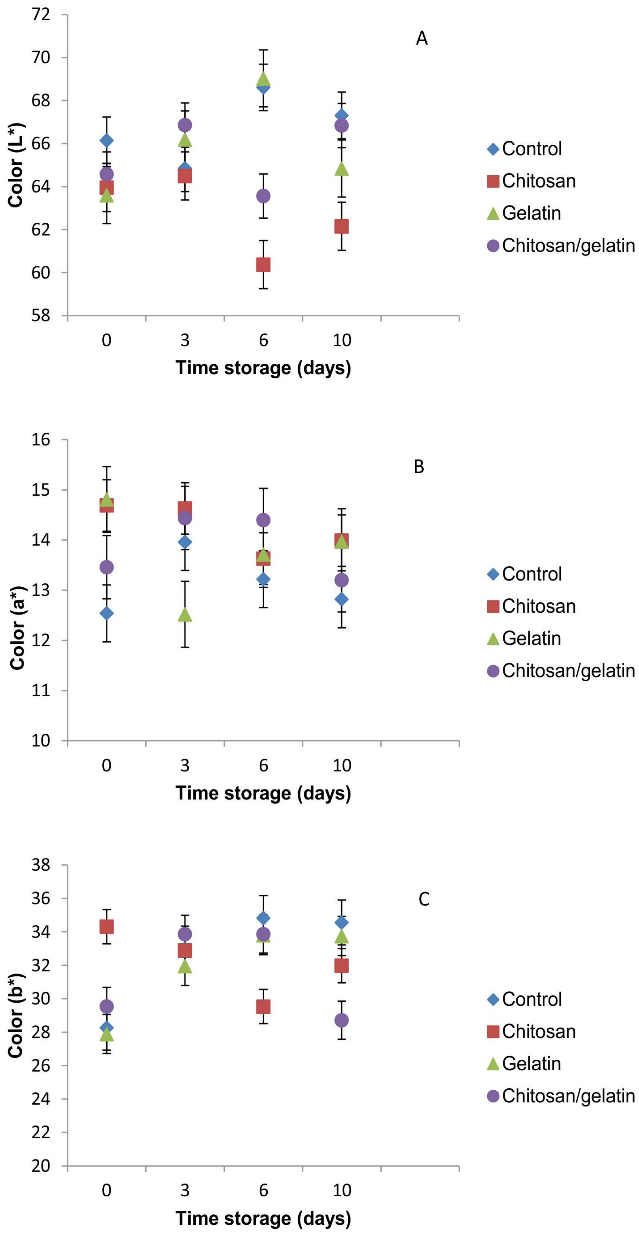

Figure 2. Color [L* (A), a* (B) and b* (C) coordinates] of minimally processed pinhões coated with chitosan, gelatin and chitosan/gelatin using the Layer-by-Layer (LbL) technique, stored under refrigeration at 4 °C for 10 days. Vertical bars represent the 95% confidence interval.

These results demonstrate that the chitosan treatment caused darkening of the minimally processed pinhões. Poverenov et al. (2014) and Vargas et al. (2006) also observed a reduction in the L* coordinate when using chitosan as a vegetable coating, as it provided greater opacity. No relationship was observed between darkening and vitamin C content.

Moreira et al. (2020) observed different behaviors in the L* values of minimally processed pinhões as a function of the applied edible coating.

Regarding time, oscillation of a* values was observed, with no significant differences in values between the first and last storage days ($p \geq 0.05$) (Figure 2B). There was no significant difference ($p \geq 0.05$) in values between treatments at the end of storage (Supporting information F). The results showed that the pinhões had a tendency to red color. The same behavior was observed by Moreira et al. (2020) when assessing the coloration of minimally processed pinhões coated with chitosan and xanthan gum/clove essential oil.

When evaluating the b* coordinate, which reflects the variation between yellow and blue, the control and gelatin-coated samples showed a significant increase ($p \leq 0.05$) during storage. As to the pinhões coated with chitosan and chitosan/gelatin, the values fluctuated, with a maintenance trend (Figure 2C). At the end of storage, the chitosan/gelatin-coated pinhões showed significantly ($p \leq 0.05$) the lowest values in relation to the other treatments, that is, a reduction in the intensity of the yellow color (Supporting information G). Moreira et al. (2020) observed no influence of time or coatings on b* values in minimally processed pinhões.

3.2 Microbiological analysis

In all treatments there was a significant increase during storage ($p \leq 0.05$) in the fungal count of minimally processed pinhões, as shown in Figure 3A. However, the chitosan/gelatin coating caused the inhibition of fungal growth in minimally processed pinhões until the sixth day of storage. Among the main fungi that affect pinhão seeds are the genera *Penicillium* sp, *Trichoderma* sp, *Rhizopus* sp, *Pestalotiopsis* sp and *Cladosporium* sp. (Hennipman et al., 2017).

At the end of storage, it was observed that the gelatin-coated sample (2.28 Log CFU g^{-1}) showed significantly lower fungal growth ($p \leq 0.05$) compared to the control sample (2.8 Log CFU g^{-1}) and the chitosan-coated sample (2.64 Log CFU g^{-1}) (Supporting information H).

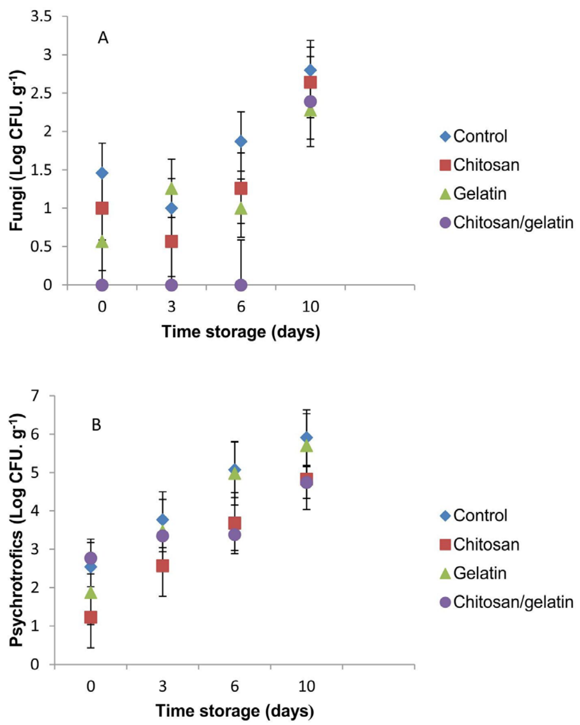

Figure 3. Quantification of fungi (A) and aerobic psychrotrophic microorganisms (B) in minimally processed pinhões coated with chitosan, gelatin and chitosan/gelatin using the Layer-by-Layer (LbL) technique, stored under refrigeration at 4 °C for 10 days. Vertical bars represent the 95% confidence interval.

Although chitosan has antimicrobial properties (Dutta et al., 2009) and contributed to the reduction in fungal growth, the presence of gelatin was more effective in this reduction, possibly due to the oxygen barrier provided by the coating. It is noteworthy that the chitosan and gelatin-based coating inhibited the fungal growth in the pinhões until the sixth day of storage; at the end of storage, the values did not differ in relation to the gelatin coating.

Moreira et al. (2020) observed no influence of coatings on fungal count (mean 4.55 Log CFU g^{-1}) in minimally processed pinhões coated with chitosan or xanthan gum/clove essential oil, obtaining higher values than those observed in this study for 9 days of storage.

Different combinations of polymers applied with the LbL technique have shown efficiency in reducing fungi in minimally processed vegetables (Treviño-Garza et al., 2017). However, Poverenov et al. (2014), when evaluating fungal growth in minimally processed melons with alginate and chitosan coating using the LbL technique, observed a greater reduction in microorganism count with the use of chitosan alone.

All treatments found a significant increase during storage ($p \leq 0.05$) in the aerobic psychrotrophic microorganism count in minimally processed pinhões, as shown in Figure 3B. However, from the third day of storage, the chitosan and chitosan/gelatin coatings caused a reduction in the growth of aerobic psychrotrophic microorganisms in relation to the control treatment.

At the end of storage, it was observed that the chitosan/gelatin sample (4.74 Log CFU g^{-1}) showed significantly lower growth of psychrotrophic microorganisms ($p \leq 0.05$) compared to minimally processed gelatin-coated pinhões (5.7 Log CFU g^{-1}) and the control sample (5.9 Log CFU g^{-1}) (Supporting information I).

The results showed a greater participation of chitosan in reducing the growth of psychrotrophic microorganisms in relation to fungi, because gelatin does not have antibacterial property (Poverenov et al., 2014).

The antimicrobial action of chitosan against bacteria may be associated with the positive charges on the molecule due to deacetylation, which allows the interaction and formation of polyelectrolytic complexes with bacterial cell surface polymers (Durango et al., 2006).

Moreira et al. (2020) also observed lower growth of aerobic psychrotrophic microorganisms when using chitosan to coat minimally processed pinhões, when compared to coating with xanthan gum/clove essential oil.

Sipahi et al. (2013), when evaluating the use of the LbL technique for the coating of minimally processed watermelon with alginate and pectin, observed a reduction in the growth of psychrotrophic microorganisms, depending on the polymer concentration used.

3.3 Sensory analysis

According to the significance level table for the triangular test (Instituto Adolfo Lutz, 2008), the minimum number of correct answers for a statistical difference at 5% significance level, for a total of 60 judgments, was associated with 27 correct answers. Thus, due to the number of hits (Table 1), there was no significant difference ($p \geq 0.05$) in the results between the coated pinhões in relation to the control sample.

Table 1. Sensory analysis by triangular test of the minimally processed pinhões coated with chitosan, gelatin and chitosan/gelatin.

Coatings	Number of evaluations	Number of hits obtained
Chitosan *versus* control	60	23^{ns}
Gelatin *versus* control	60	22^{ns}
Chitosan/gelatin *versus* control	60	26^{ns}

^{ns}not significant.

Thus, the taste and aroma of pinhões coated with chitosan, gelatin or chitosan/gelatin did not differ from control samples, which did not receive coatings. These results indicated that coatings were a conservation alternative for minimally processed pinhões.

Treviño-Garza et al. (2017) also did not observe differences in sensory characteristics of odor and flavor in relation to the control sample, when evaluating minimally processed pineapple coated with different polymers, using the LbL technique.

4 Conclusion

The production of minimally processed pinhões is an alternative presentation to the product *in natura*, which has the benefit of practicality. The application of the chitosan/gelatin coating using the Layer-by-Layer technique allowed the reduction of weight loss, fungal growth and aerobic psychrotrophic bacteria. The coating did not slow the maturation process and consequently, higher vitamin C contents were obtained. The coatings did not influence the taste and aroma of minimally processed pinhões. It is believed that minimal processing can encourage the consumption of pinhões by facilitating the preparation of the seed.

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