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APPLICATION OF BIOPOLYMERS FOR FUNGAL PATHOGEN CONTROL IN THE STORAGE OF FRUITS

Diyana ALEKSANDROVA[1], Sashka SAVCHOVSKA[1], Gabor ZSIVANOVITS[2]

[1]Agricultural Academy - Sofia, Fruit Growing Institute - Plovdiv, Ostromila 12, Plovdiv, Bulgaria
[2]Agricultural Academy - Sofia, Institute of Food Preservation and Quality, Plovdiv, Bulgaria

Corresponding author email: aleksandrova_diyana@abv.bg

Abstract

The edible coatings of biopolymers such as alginate and chitosan offer promising opportunities for improving the storage of fruits by reducing losses due to fungal diseases. However, it is important to conduct further research to optimize the conditions for their application and ensure their safety and effectiveness in real-world conditions. Nectarine fruits were artificially inoculated with Monilinia spp. and, one day later, coated with 1% chitosan and alginate films. Characterisation of the differently coated fruit was traced during refrigeration. The inhibitory effect of coatings was monitored by measuring the diameter of the lesions on fruits over ten days, and the water loss by measuring milligrams of the fruits. The highest percentage of repression of fungal disease was observed in chitosan coating. The alginate coating has shown a positive effect on fruits during storage to save water contained and extend shelf-life time. The results obtained were compared with the control fruits which were not-application of biopolymer film.

***Key words**: Monilinia spp. chitosan, alginate, peach, post-harvest, waste reducing.*

INTRODUCTION

The peach (*Prunus persica* L.) is a preferred fruit by consumers due to its exotic taste and nutrient-rich fruits. Peaches contain several antioxidant compounds, including vitamins, phenols, carotenoids, and organic acids (Hussain et al., 2021). Fresh stone fruits are susceptible to decay, which several pathogenic fungi can cause (Lopez-Reyes et al., 2013). The main pathogens that infect peach fruits include *Botrytis cinerea* (Pers.: Fr.), *Penicillium* expansum (Link), *Rhizopus* stolonifer (Ehrenb. Fr.), and *Monilinia* spp. (Karabulut & Baykal, 2002; Zhang et al., 2007). Peach and nectarine production is stable and has been growing in recent years. The main problem is during the storage period and transportation of fruits due to their rapid decay and high transpiration rate at room temperature, which hinders their export (Lurie and Crisosto, 2005; Hussain et al., 2021). Losses caused by various fungal pathogens during fruit storage can reach up to 50% under favourable conditions; losses are significantly lower with synthetic fungicide treatments, around 10% of fruit production (Margosan et al., 1997). The global trend is to seek new alternatives to synthetic fungicides that can successfully control diseases during storage while having no side effects on human health (Johnson & Sangchote, 1994). The need to develop new methods for controlling fruit decay is driven by the excessive use of fungicides during vegetation, and the ecological plasticity of phytopathogens reduces the effectiveness of used fungicides (Lopez-Reyes et al., 2013). Improving fruit production quality by extending shelf life, reducing decay rates, and providing minimally processed fruits that are safe for human health is possible through the use of biocompatible packaging. Biological fruit packaging has been researched and developed for healthier preservation (Wu et al., 2022; Hadimani et al., 2023).

Fruit coatings are made from edible polymer materials derived from renewable sources, which are by-products of the food industry. They are used as edible packaging to extend shelf life and improve the quality of food products by adding active ingredients such as antioxidants, vitamins, and antimicrobial agents (Hosseinnejad & Jafari, 2016).

Chitosan is a natural polysaccharide biopolymer derived from chitin. Depending on the degree of deacetylation, chitosan can have several amino groups. This polycationic nature imparts antimicrobial properties to chitosan, which facilitates interaction with negatively charged microbial cell walls and cytoplasmic membranes. These interactions lead to reduced osmotic stability, membrane disruption, and eventual leakage of intracellular components (Ma et al., 2008; Banerjee et al., 2010). Cited as the second most widespread polysaccharide in nature, chitosan possesses a range of positive qualities, including being biodegradable and non-toxic to humans and the environment, as well as biocompatible and biofunctional (Sharma et al., 2021). The biodegradation of the chitosan depends on the concentration of the nutrients and on the microorganisms in the coated product (Nakashima et al., 2005). Based on other references the chitosan is stable for the first 30 days and the process of degradation is around 100-200 days, depending on the used additives as well (Schnabl et al., 2024).

Among the polysaccharides used as edible packaging, sodium alginate (NaAlg), derived from marine brown algae, also finds application. This biopolymer is of interest as a potential coating component due to its unique colloidal properties and its ability to form strong gels alone or in combination with aqueous solutions. Alginate extends the shelf life of fruits by reducing diffusion processes and maintaining high concentrations of active molecules on the food surface. Alginate-based coatings provide additional protection to fruits by acting as a semi-permeable barrier to reduce weight loss, gas exchange, respiration, and the rate of oxidative reaction (Rojas-Grau et al., 2009). The existing literature says, the biodegradation of the alginate is much faster than the chitosan's. The weight loss of the alginate in the first 30 days is near to 50% and the fully degradation is around 45 days (Dalal et al., 2023).

This study aimed to determine the influence of the biopolymers chitosan and alginate after artificial inoculation of peach fruits with the pathogen *Monilinia* spp.

MATERIALS AND METHODS

The infected nectarine fruits were selected with visual symptoms of rot caused by fungal pathogens. A piece of infected tissue was placed on Potato Dextrose Agar (PDA) and the Petridis dish was incubated at a controlled temperature. The pathogen was isolated by standard phytopathological methods. The single colony of the pathogen *Monilinia* spp. was cultured on PDA in a controlled environment maintained at a constant temperature of 23°C, to ensure optimal fungal growth and sporulation.

Nectarine fruits of the 'Andrews' cultivar were selected as the experimental fruit. Fruits were harvested directly from the field at pre-consumer maturity, ensuring uniformity in ripeness. To eliminate surface contaminants, the nectarines were surface sterilized with 70% alcohol.

The inoculation was performed using a standardised spore culture of the *Monilinia* spp. pathogen. The spore concentration was precisely determined using a hemocytometer, adjusting the suspension to a concentration of 10^6 conidia per millilitre. A precise volume of 25µl of this spore suspension was injected into each nectarine to begin infection. The inoculation fruits were stored in a refrigerator at 4°C for 24 hours.

The biopolymers chitosan and alginate were used at a 1% concentration. The infected nectarine fruits were dipped in a solution of the biopolymers each fruit single.

The development of fruit rot was recorded dynamically every two days. This was achieved by measuring the diameter of the rot-affected area on each fruit. Measurements were conducted using a calliper to ensure the accuracy and consistency of the data. These dynamic measurements allowed tracking the progression of rot over time and evaluating the effectiveness of biopolymer coatings (chitosan and alginate) in delaying or preventing the development of the pathogen *Monilinia* spp.

For control variants were used inoculation fruits without the application of biopolymers. For data significance, 20 fruits per replicate were used for each treatment variant (control, chitosan, and alginate).

The percentage inhibition of mycelial growth was calculated using the formula:

$\text{Inhibition}\% = \frac{(\text{dc}-\text{dt})}{\text{dc}} \cdot 100$, where

dc = the average diameter of the fungal colony in the control variant, dt = the average diameter of the fungal colony of the treatment variant. In

addition, the weight loss of uninfected fruits was measured. The values were presented as a percentage during observation.
The data obtained was analysed statistically using the SPSS 19.0 program. The data was subjected to Duncan's Multiple Range Test (MRT) at P≤0.05.

RESULTS AND DISCUSSIONS

To investigate the efficacy of biopolymer coatings on nectarines, it was necessary to isolate and cultivate the fungal pathogen responsible for the infection. Samples were isolated from infected nectarine fruits, from which the fungal pathogen *Monilinia* spp. was isolated. The isolation was performed by excising infected tissue from the fruit and placed on a potato dextrose agar (PDA) medium in a Petri dish (90 mm). The samples were incubated in a controlled environment maintained at a constant temperature of 23°C. This temperature was optimal for the growth and sporulation of *Monilinia* spp. During 10 days, colonies of *Monilinia* spp. developed on the PDA. Single colony were transferred in Petri dish to obtain a pure culture of the pathogen. This process of transferring single colonies was repeated three times that the culture was free of other contamination.
The pure culture of *Monilinia* spp. was used to inoculate nectarines. The coated biopolymers chitosan and alginate were applied 24 hours after inoculation. The control fruits, which were not coated with biopolymers, were also inoculated with the pathogen to provide a baseline for comparison. This controlled experimental assessment evaluated the inhibitory effect of chitosan and alginate on the growth of *Monilinia* spp. on nectarines.
The results (Table 1) demonstrated the growth of pathogens over ten days in nectarines application with alginate, chitosan, and an untreated control group. After two days, the infected area was relatively small in all variants, with alginate-treated fruits showing a slightly lower infection level (6.1 mm) compared to chitosan (7.8 mm) and the control (6.9 mm). By the fourth day, the infection spread further, with alginate (10.5 mm) and the control (10.2 mm) showing similar levels of fungal growth, while chitosan-treated fruits exhibited a slightly lower infection rate (8.2 mm).

Table 1. Inoculation of nectarine fruits (*Monilinia* spp.) by application with biopolymers chitosan and alginate

Variant	**Infected area of the fruits (mm)**				
	After two days	After four days	After six days	After eight days	After ten days
Alginate	6.1±1.68a	10.5± 5.50 a	13.2± 10.06 a	17.7 ±7.93a	21.5± 7.47 a
Chitosan	7.8 ± 2.73a	8.2 ± 4.69a	10.6± 9.00 a	13.5 ± 7.09a	12.7± 6.29 a
Control	6.9 ± 6.37a	10.2± 7.47 a	14.7± 12.51 a	21.5± 12.70 a	21.7 ± 12.20a

As the experiment progressed, differences between the variants became more evident. After six days, the control fruits showed the highest infected area (14.7 mm), while alginate (13.2 mm) and chitosan (10.6 mm) displayed lower infection levels. By the eighth day, the infection had advanced significantly in the control group (21.5 mm), whereas chitosan-treated fruits maintained a lower infection level (13.5 mm), suggesting a stronger inhibitory effect. Alginate coated fruits showed an intermediate infection level (17.7 mm).
After ten days, the control fruits had the highest infection area (21.7 mm), while alginate-treated fruits exhibited a slightly lower infection level (21.5 mm). Chitosan treated nectarines had the lowest infection area (12.7 mm), indicating its antifungal effect over time. These results confirmed that both alginate and chitosan slowed the infection, with chitosan being the more effective in limiting fungal growth on nectarines.
The statistical analysis of variance ANOVA allowed for the assessment of whether the observed differences in infection area among alginate, chitosan, and control groups were statistically significant. The result of experiment showed of the same ("a") values

that no significant differences were detected among the experimental variants. This indicated that, despite observable variations in mean infection areas, these differences were non- statistically significant.
The variability in fungal growth pathogen was calculated trought the standard deviation values.
On day 2, alginate had the lowest standard deviation (±1.68), while the control showed the highest (±6.37), indicating that infection spread more uniformly in the treated fruits. By day 4, standard deviations increased, with chitosan (±4.69) showing the least variability, suggesting that it provided a more stable antifungal effect.
The devepompent of *Monilinia* spp. in the control group showed the highest variability, reaching ±12.51on day 6 and ±12.70 on day 8, while alginate-treated fruits had slightly lower deviations (±10.06 and ±7.93, respectively). Chitosan consistently showed the lowest variation, with ±9.00 on day 6 and ±7.09 on day 8, indicating that it maintained a more uniform inhibitory effect.
By day 10, the control had the highest standard deviation (±12.20), followed by alginate (±7.47), while chitosan-treated fruits showed the most stable results (±6.29). These trends also concirmed that, although all biopolymers slowed infection, chitosan provided the most consistent protection against fungal growth.
The graph (Figure 1) displayed the dynamics of *Monilinia* spp. inhibition by alginate and chitosan across five measured in each two days of development of mycelia. The percentage of inhibition by alginate started with a negative value (-2.94%). The maximum inhibition reached 17.67% at the fourth measure but decreased to 0.92% at the last observation.

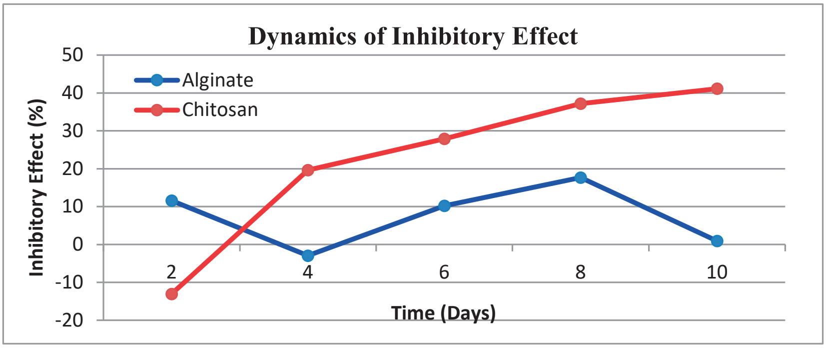

Figure 1. Inhibition of mycelia growth relative to control variant (%)

The results of edible film chitosan showed a progressive increase in inhibition, the value started with a negative percentage (-13.04%) but in the second and the next measures increased significantly (41.11%) the pressure of the pathogen. The biopolymers demonstrated more stable and effective inhibition of *Monilinia* spp. compared to alginate. Chitosan showed greater potential for controlling the brown rot caused by the fungus pathogen *Monilinia* spp.
Numerous authors have investigated the influence of biopolymers, including chitosan and alginate, on various fungal pathogens. The significant inhibition report (Romanazzi et al., 2017) of inoculation strawberries with *Botrytis cinerea* when treated with chitosan. Confirms have been reported from El Ghaouth (1992) was studied the antifungal activity of chitosan against pathogens *Botrytis cinerea* and *Rhizopus stolonifer*. The effectiveness of chitosan was reported with a coating enriched with cinnamon oil against *Monilinia fructicola* (Duan et al., 2019).
It has also been reported that alginate has a positive effect on successfully controlling pathogens during the storage period. For example, citrus fruits can be inoculated with *Penicillium digitatum* (Rojas-Graü et al., 2008), and sweet cherries can be inoculated with *Monilinia fructicola* (Gol et al., 2013).
Data obtained showed the weight loss (in percentages) of fruits treated with alginate and chitosan, as well as a control variant, the

nectarine fruits were refrigerated storage (Table 2).
The expected progressive increase in weight loss, reflecting the natural dehydration of fruits during storage (3.53%, 6.63%, 7.61%) in the control variant, was observed in a significantly lower weight loss in fruits coated with chitosan and alginate compared to the control group fruits. This indicated that the chitosan and alginate coating effectively reduced the rate of dehydration, by creating a barrier that limited moisture transfer, which was confirmed by the data in Table 2, and was consistent with the research of the author (Momchilova, M. et al., 2021).

Table 2. Relative weight loss of nectarine fruits covered with different edible coatings during storage

Variant	**Weight Loss (%)**				
	I	II	III	Sum	Average SD
Alginate	3.02±0.37	1.94±0.22	3.80±0.50	8.77	±0.37
Chitosan	5.73±0.79	2.76±5.03	1.36±4.35	9.85	±3.39
Control	3.53±7.43	6.63±5.43	7.61±1.55	17.77	±4.80

*SD - Standard Deviation

Edible films prevented moisture losses during postharvest storage therefore, water vapour uptake and water vapour permeability were important parameters to characterize the biopolymers used. Water vapour uptake of chitosan films decreased during storage at room temperature but increased during storage at low temperatures in a freezer and refrigerator. Thinner chitosan films exhibited lower water vapour permeability (Kerch G. & Korkhov V., 2011). Chitosan coatings, effectively delay respiration, reduce weight loss, and extend the shelf life of fruits and vegetables during postharvest storage. Chitosan-based edible coatings impact shelf life, microbiological quality, and biochemical processes, supplementing previous reviews (Dhall, 2013; Shiekh et al., 2013). Alginate treatment was an effective tool to delay the postharvest ripening process of sweet cherries. This resulted in reduced color changes, losses in acidity and firmness, and respiration rate. Furthermore, alginate treatment maintained a higher concentration of total phenolics (Díaz-Mula et al., 2012). The combination of thymol with sodium alginate films offers an alternative to traditional biocompatible wrap for packaging fresh-cut fruits. This composite film holds promise, but its practical implementation at an industrial level necessitates further advancements and optimization (Chen et al., 2021).
The fruits cover with alginate, the standard deviation across different storage periods was relatively low (±0.37) on the fourth day, the value ±0.22 on the sixth day, and 0.50 on the eighth day. The lowest value (±0.22) on the sixth day indicated minimal variation in weight loss measurements, suggesting that alginate provided a uniform and stable effect on moisture retention in the fruit. The slightly higher deviations on the fourth and eighth days still remained low, implying that alginate acted consistently across all samples, reducing water loss.
In contrast, nectarines treated with chitosan exhibited much higher standard deviations ± 0.79 on the fourth day, ± 5.03 on the sixth day, and ±4.35 on the eighth day. While the fourth day showed relatively low deviation, indicating some stability, the sixth and eighth days demonstrated significantly greater variation. The high deviations (±5.03 and ±4.35) suggested that chitosan coating wasn't uniform and infected area of fruits were measured with high variation.

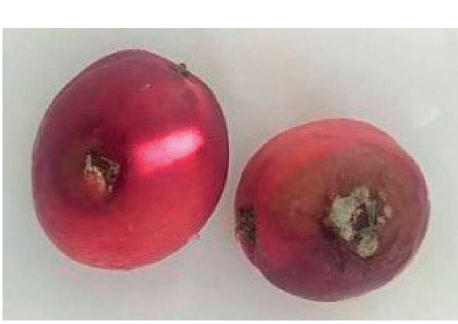

Figure 2. Nectarine fruits application with alginate

The control group, which was not treated with any coating, exhibited the highest standard deviations, indicating extremely large differences in weight loss among individual fruits. On the fourth day, the standard deviation reached ±7.43 the highest recorded value in the study suggesting that while some samples retained moisture, others lost significant amounts of water. On the sixth day, the deviation decreased slightly to ±5.43 but remained high, while on the eighth day, it dropped further to ±1.55, suggesting that over time, the rate of weight loss became somewhat more uniform, although still more variable than in the treated groups.

The total weight loss of 17.77% emphasizes the need for protective coatings to extend the shelf life of nectarines.

Chitosan results showed a larger initial weight loss (5.73%) compared to alginate and the control variant. This may be due to the slower formation of a protective layer by chitosan or its greater permeability in the early stages of storage. However, weight loss decreases significantly during the second (2.76%) and third (1.36%) periods, indicating that chitosan becomes more effective over time. The total weight loss of 9.85% was higher than that of alginate but still significantly lower than the control group. The effectiveness of biopolymers chitosan and alginate was confirmed in many researches (Gol N. B., 2013; Petri S. & Sharma R. R., 2021).

Figure 3. Nectarine fruits application with chitosan

Comparing the average standard deviations across the three treatment ±0.37 for alginate, ±3.39 for chitosan and ±4.80 for the control varint it was evident that alginate provided the most stable coating, ensuring minimal variation in weight loss. Chitosan showed greater variability, potentially due to uneven distribution or interactions with the fruit surface, while the control group experienced the most unpredictable weight loss, confirming the lack of a protective barrier.

The coating fruits with alginate showed effectiveness in reducing weight loss. The low weight loss during the first two periods of measuring (3.02%, 1.94%) indicates that alginate quickly forms an effective barrier that slowed down moisture evaporation. The total weight loss of 8.77% is significantly lower than the control variant, confirming the protective properties of alginate.

These findings aligned with previous scientific studies analyzing the effects of coatings on fruit water loss. For example, research by Valero et al. (2013) on plums treated with chitosan also reported high standard deviations in later storage stages, attributed to uneven coating distribution and varying water loss rates. Olivas & Barbosa-Cánovas (2005) found that alginate coatings on apples and pears significantly reduced standard deviation in weight loss, forming a more homogeneous barrier against evaporation. Similarly, Dhall (2013) reported that control samples without coatings exhibited the highest deviations, consistent with observations in the present study.

CONCLUSIONS

The chitosan coating demonstrated the least mycelia growth of inoculated nectarines, although the differences compared to the other variants were not statistically significant.

The best variant of inhibition of mycelia growth reached 41.11% at the end of the observation period, compared to the alginate which was measured at 0.92%.

The biopolymer alginate was most effective in reducing weight loss.

Based on the experimental results and on the biodegradability of the applied biopolymers both of them were capable of short time fruit storage but with different time stability and other advantages.

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