Investigation note

Chitosans and chitosan-sodium octanoate composite reduce strawberry rot in postharvest

Sigifredo López-Díaz[1]
Ma. Guadalupe Sandoval-Flores[2]
Luis Enrique Flores-Pantoja[3]
Rafael Jiménez-Mejía[3]
Gustavo Santoyo[4]
Pedro Damián Loeza-Lara[3§]

[1]Interdisciplinary Research Center for Integral Regional Development-Michoacán Unit. Jiquilpan, Michoacán, Mexico. CP. 59510. Tel. 353 5330218, ext. 82923. (slopezd@ipn.mx). [2]Multidisciplinary Center for Studies in Biotechnology-Faculty of Veterinary Medicine and Zootechnics-Michoacan University of San Nicolás de Hidalgo. Morelia, Michoacán, Mexico. CP. 58893. Tel. 443 2958029, ext. 111. (lupitaflores96@outlook.com). [3]Bachelor of Food Genomics-University of La Ciénega of the State of Michoacán de Ocampo. Sahuayo, Michoacán, Mexico. CP. 59103. Tel. 353 5320762, ext. 1420. (leflores@ucienegam.edu.mx; rjimenez@ucienegam.edu.mx). [4]Institute of Biological Chemical Research-Michoacana University of San Nicolás de Hidalgo. Morelia, Michoacán, Mexico. CP. 58030. Tel. 443 3265788, ext. 125. (gsantoyo@umich.mx).

[§]Corresponding author: pdloeza@ucienegam.edu.mx.

Abstract

Strawberry (*Fragaria* x *ananassa*) is an exquisite food, which provides health benefits, making it the berry with the highest production and export in Mexico. However, it is highly perishable, susceptible to postharvest damage, mainly by *Botrytis cinerea* and *Rhizopus stolonifer*, among others. The use of pesticides in preharvest is the control strategy of these pathogens; however, pesticides are documented to harm human health and the ecosystem, showing the need to explore friendly alternatives. Reagent grade chitosan (QGR) is an innocuous polymer with widely reported antifungal activity, while sodium octanoate (8:0) (OS) also has this property; however, so far it is unknown whether commercial grade chitosan (QGC) (the most suitable candidate to conduct a larger commercial study, due to its cost), available in Mexico, has the same effect. Therefore, the objective of this research was to evaluate the effectiveness of QGR and the QGR-OS composite in the protection of strawberry in postharvest and compare it with that of QGC and that of the QGC-OS composite. The compounds were sprayed on the fruits and incubated simulating export conditions. The results showed significant reduction in the severity and incidence of postharvest strawberry fungal diseases after the application of QGR, QGC and QGR-OS, QGC-OS, but not that of OS applied alone. QGC and the QGC-OS composite are excellent candidates for use in a larger commercial study.

Keywords: innocuous alternatives, fungal diseases, antifungal polymers.

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Strawberry (*Fragaria* x *ananassa* Duch.) is a berry with exquisite flavor and aroma that provides health benefits such as heart protection and blood pressure balance, in addition to having antioxidant and anticancer activity, among others (Lombardi *et al*., 2020). These effects are related to the content of phenolic compounds that it has, such as anthocyanins that slow cellular aging. It also contains minerals such as iron, phosphorus, copper, magnesium and vitamins A, E, K and C, mainly (Forbes-Hernández *et al*., 2017).

Unfortunately, this berry is highly perishable, particularly during the postharvest stage, as it is susceptible to rot and infection by pathogenic fungi. Grey mold and soft rot are diseases caused by *Botrytis cinerea* (Pers.) and *Rhizopus stolonifer* (Ehrenb.), respectively, which are considered the leading cause of postharvest strawberry rot (Feliziani and Romanazzi, 2016). However, other important pathogens have been reported at this stage such as *Mucor* spp., *Colletotrichum* spp., *Penicillium* spp., *Phytophthora* spp., and *Fusarium* spp. (Lopes *et al*., 2014; Feliziani and Romanazzi, 2016; Arceo-Martínez *et al*., 2019).

The rot control strategy is based on the application in field of synthetic chemical fungicides during crop growth, for instance, Captafol® and Diclorán®. However, numerous studies show that continuous exposure to pesticides in general is associated with adverse effects on human health and pollution of the ecosystem (Curl *et al*., 2020; Hassaan and El Nemr, 2020). The above shows the need to study friendly alternatives for the control of pathogenic fungi.

Among the friendly alternatives is the reagent grade chitosan (QGR), an innocuous biopolymer, with antifungal activity on the main postharvest strawberry pathogens (Lopes *et al*., 2014; Feliziani and Romanazzi, 2016; Lizardi-Mendoza *et al*., 2016; Romanazzi *et al*., 2017; Arceo-Martínez *et al*., 2019; Mejdoub-Trabelsi *et al*., 2019). Sodium octanoate (8:0) (OS), harmless fatty acid salt, with antifungal activity on strawberry pathogens, also stands out (Liu *et al*., 2008; Pohl *et al*., 2011; Sandoval *et al*., 2018; Yoon *et al*., 2018). Additionally, the chitosan-sodium octanoate composite (QGR-OS), which showed greater protection of fruits inoculated with *B. cinerea*, stands out (Sandoval *et al*., 2018).

The above results show the potential of these molecules and their composites to be used in the control of fungal diseases of strawberry in storage. However, so far, the effect of the commercial grade chitosan (QGC) available in Mexico has not been reported, which is the most suitable candidate to conduct a study at the commercial level, since it is 25 times cheaper than QGR. Therefore, the objective of this research was to assess the effectiveness of QGC and the QGC-OS composite in the control of strawberry rot in postharvest and compare this effectiveness with that of QGR and the QGR-OS composite.

In the present study, strawberry fruits var. Frontera, obtained from the company Zarzara Biofarm SA de CV, located in Tangancícuaro, Michoacán. The fruits were selected based on the absence of damage and evident infection by microorganisms and level of ripening (2/3 parts of the fruit were red). The fruits were used in the experiment on the same day of harvest (Romanazzi *et al*., 2013). medium molecular weight QGR (190 to 300 kDa) was obtained from Sigma-Aldrich®, Mexico, while medium molecular weight QGC (50 to 190 kDa) was obtained from Future Foods® SA de CV, Mexico.

A solution of each polymer was prepared at a concentration of 20 mg ml^{-1}, in distilled water and 1% acetic acid (J. T. Baker®, Mexico). These solutions were kept in constant agitation for 24 h. Subsequently, the pH of the solution was adjusted to 5.6 with NaOH 1 N (J. T. Baker®, Mexico) and the solution was sterilized at 120 °C for 15 min (modified from Liu *et al*., 2007). On the other hand, OS was obtained from Sigma-Aldrich®, Mexico. Fatty acid was prepared at a concentration of 50 mg ml^{-1} in distilled water, which was sterilized by filtration using membranes of 0.22 µm pore diameter (Merck Millipore®, USA) (modified from Liu *et al*., 2008).

The composites were prepared as follows: once the two polymers and the fatty acid were sterilized, it was mixed with QGR and QGC. The composites were kept in constant stirring at room temperature (25 ±2 °C). Prior to use in bioassays, the composites were stirred vigorously for 5 min in Vortex (Sandoval *et al*., 2018).

All treatments were sprayed at the following concentrations: QGR and QGC (15 mg ml^{-1}), OS (0.49 mg ml^{-1}), QGR-OS and QGC-OS (15-0.49 mg ml^{-1}) (Sandoval *et al*., 2018). An absolute control was established: sterile distilled water and a negative control: 1% acetic acid, pH 5.6. After applying the treatments, the strawberries were left to dry in laminar flow cabinet (CHCbiolus®, Mexico) for 1.5 h. They were then placed in sterile plastic boxes (30 x 32 cm) and stored for 7 days at 2 ±2 °C, 95-98 HR. After this period, the strawberries were placed at room temperature (25 ±2 °C, 95-98 HR) for 3 days to represent the storage conditions of the strawberry for export.

After this time, the severity and index of the diseases were determined. Five replications of 30 strawberries were used for each treatment (150 fruits per treatment). The infections that subsequently appeared corresponded to the natural inoculum of the fruits (Romanazzi *et al*., 2013). The severity of disease damage was obtained according to the empirical scale of Romanazzi *et al*. (2013), which uses six levels: 0, healthy fruit; 1, 1-20% of infected fruit; 2, 21-40% of infected fruit; 3, 41-60% of infected fruit; 4, 61-80% of infected fruit; 5, more than 81% of the infected fruit.

This scale allowed the calculation of the McKinney index, which is an infection index that shows variable values from 0 (no disease) to 100% (maximum level of the disease) (McKinney, 1923). Isolated fungi were identified culturally and by microscopic morphology according to Barnett and Hunter (1998); Maas (1998). The treatments were applied under a completely random experimental design. The data obtained (n= 150 per treatment) were transformed with the function $\sqrt{x} + 0.5$, were subjected to an analysis of variance (Anova $p< 0.05$) and a comparison of means with the Tukey test ($p< 0.05$) was performed. The statistical program SPSS-IBM Statistics version 25 was used.

Control of strawberry rot in postharvest by chitosans, OS and their composites

In the present study, strawberry fruits treated with QGR (15 mg ml^{-1}), QGC (15 mg ml^{-1}) and QGR-OS composites (15-0.49 mg ml^{-1}) and QGC-OS (15-0.49 mg ml^{-1}) showed significant reduction ($p< 0.05$) in infections caused by pathogenic fungi, which was reflected in the severity of fungal diseases and in the McKinney index, compared to the negative control. Treatments with QGR and

QGC showed McKinney indices of 7 and 38.8%, respectively; while the negative control showed an index of 52%. On the other hand, the application of OS (0.49 mg ml^{-1}) did not show effectiveness (index of 55.3%) since an infection greater than that in the negative control was observed ($p < 0.05$). Likewise, the QGR-OS and QGC-OS composites showed indices of 6 and 19.8%, respectively, showing differences with the negative control ($p < 0.05$) (Table 1, Figure 1).

Table 1. Reduction of the severity of fungal diseases in strawberry fruits treated with the compounds after 10 days in storage.

Treatments (mg ml^{-1})	Degree of severity (damage scale 0-5)	McKinney Index (%)
QGR (15)	1	7 b
QGC (15)	2	38.8 d
OS (0.49)	3.7	55.3 f
QGR-OS (15/0.49)	1	6 a
QGC-OS (15/0.49)	1	19.8 c
Absolute control*	3.6	52 e
Negative control¶	3.6	52 e

Values with different letter in each column are different according to Tukey ($p < 0.05$). *= Strawberry fruits with sterile distilled water; ¶= Strawberry fruits treated with 1% acetic acid, pH= 5.6.

Figure 1. Effect of the application of chitosans and OS in the reduction of the severity of fungal diseases in strawberry fruits. A) QGR (15 mg ml^{-1}); B) QGC (15 mg ml^{-1}); C) OS (0.49 mg ml^{-1}); D) QGR-OS (15-0.49 mg ml^{-1}); E) QGC-OS (15-0.49 mg ml^{-1}); (F) absolute control; and (G) negative control.

Numerous studies have demonstrated the ability of chitosan to inhibit the mycelial growth of pathogenic fungi, which is explained by the cationic nature of the polymer that affects the synthesis of the cell wall of pathogens, as well as the structure of the membrane. This mechanism is exerted by establishing electrostatic attractions with molecules that have anionic groups. This causes an imbalance in the synthesis of the cell wall and the pore formation in the membrane, with the consequent outflow of the cytoplasmic content and the subsequent death of the cell (Verlee *et al*., 2017; De Oliveira *et al*., 2020).

The combinations of QGR-OS and QGC-OS were the best treatments, compared to the compounds applied alone, since the former showed a fruit protection of 94%, while the latter recorded a protection of 80.2% (Table 1, Figure 1).

Previous studies show the properties of chitosan to form a protective film and be an excellent matrix for various compounds such as food additives and secondary metabolites, with the aim of increasing the biological effect of the polymer, increasing the nutritional value of the fruits and their microbiological quality (Hernández-Muñoz *et al*., 2008; Rodríguez-Romero *et al*., 2019). Synergy is an effect that results from the combination of two compounds, which may differ from the effects of each of the parts (Ryabushkina, 2005), thus, according to the results obtained (Table 1), it is observed that the OS per se has no effect against the pathogens evaluated; nevertheless, the synergistic effect it provided as part of the composites tested is statistically significant, which coincides with Sandoval *et al*. (2018) report, who observed a greater protective effect of OS when forming the QGR-OS composite.

Likewise, a greater antifungal effect of QGR (medium molecular weight) is highlighted compared with QGC (low molecular weight), both used alone and in combination with OS. It has been reported that the antimicrobial effect of chitosan depends on different physicochemical characteristics, including molecular weight (Rodríguez-Pedroso *et al*., 2009). The above results are consistent with those in Rodríguez *et al*. (2016), who demonstrated that medium molecular weight chitosan has a greater effect on the radial growth of the fungus *Bipolaris oryzae*, causal agent of spotted grain in rice (*Oryza sativa*).

Additionally, it is important to note that, although QGR and the combination of QGR-OS turned out to be the best treatments, QGC and the QGC-OS composite also showed favorable results, suggesting that both could be the ideal candidates to conduct a study at a commercial level, since, in addition to the effect observed on reducing the severity, the QGC available in Mexico is 25 times cheaper than QGR. Finally, it is important to mention that the fungi isolated from the infected fruits corresponded to *B. cinerea* and *R. stolonifera*, which coincides with those reported by Feliziani and Romanazzi (2016).

Conclusions

The present study shows that QGC reduced the severity of infections in strawberry fruits in storage, caused by pathogenic fungi (*B. cinerea* and *R. stolonifer*). It also reveals that the combination of QGC-OS was even more effective in protecting the fruits than the QGC applied alone. The above results suggest that both QGC-OS and QGC could be used in a commercial study to determine the ability of chitosan to maintain control of strawberry fungal diseases in postharvest and thereby improve the microbiological quality of fruits for export.

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