

# Chitosan Treatment for the Control of Postharvest Decay of Table Grapes, Strawberries and Sweet Cherries

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## ABSTRACT

Table grapes, strawberries and sweet cherries are perishable fruits and can be affected by different diseases, both in the field and even more during postharvest storage. The main decay is gray mold (caused by *Botrytis cinerea*), which infects all three fruit, and Rhizopus rot (induced by *Rhizopus stolonifer*), which attacks mainly strawberry, at times sweet cherries, and rarely table grapes. Moreover, sweet cherries can suffer heavy losses mainly by brown rot (due to *Monilinia* spp.), and to a lesser extent, by blue mold (caused by *Penicillium expansum*, at times this can infect table grapes and strawberries, too), Alternaria rot, and Cladosporium rot (induced by *Alternaria alternata* and *Cladosporium* spp., respectively). Table grapes, strawberries and sweet cherries are often cold stored at 0°C soon after harvest, to retain quality, delay senescence, and reduce decay development. In several countries the use of synthetic fungicides after harvest is not allowed or there is a very short list of approved active ingredients. Therefore, together with consumer demand for food free of pesticide residues, the use of alternative means to control postharvest decay of fruit has gained increasing interest. Among these, chitosan has been identified as having the properties of an ideal coating for fruit. Preharvest and postharvest chitosan treatments of table grapes, strawberries and sweet cherries reduce their decay in the field and during storage, with the best performance at the highest tested concentration (usually 1%). Chitosan-based commercial products are available, and they have shown the same effectiveness as the biopolymer dissolved in an acid solution. Chitosan has a double mechanism of action: it reduces the growth of decay causing fungi, and it induces resistance responses in host tissues. With this double effectiveness, chitosan can be considered as the first compound of a new class of plant protection products.

**Keywords:** coating, induced resistance, *Fragaria × ananassa*, *Prunus avium*, *Vitis vinifera*

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## INTRODUCTION

Commodities like table grapes (*Vitis vinifera* L.), strawberries (*Fragaria × ananassa* Duch.) and sweet cherries (*Prunus avium* L.) are perishable and can be infected by different pathogens, both in the field and even more so during postharvest storage. The main decay are gray mold (caused by *Botrytis cinerea* Pers.), which infects all three fruit, and Rhizopus rot (induced by *Rhizopus stolonifer* (Ehrenb.) Vuill.), which attacks strawberries and sweet cherries, and rarely table grapes (Pearson and Goheen 1988; Ogawa *et al.* 1995; Maas 1998). Moreover, sweet cherries can suffer heavy losses mainly by brown rot, which is caused by three different species of the genus *Monilinia* (*M. laxa* (Aderh. and Ruhl.) Honey, *M. fructigena* (Aderh. and Ruhl.) Honey and *M. fructicola* (Winter) Honey), and also, to a lesser extent, by blue mold (caused by *Penicillium expansum* Link, which can infect at times table grapes and strawberries too), Alternaria rot (*Alternaria alternata* (Fr.:Fr.) Keissl.), and Cladosporium rot (*Cladosporium* spp.). Table grapes, strawberries and sweet cherries are often cold stored at 0°C soon after harvest, so as to retain fruit quality and delay senescence, and thus reduce decay development. A fruit that shows a delayed ripening is less

susceptible to decay (Lougheed *et al.* 1998). Table grapes can be cold stored for up to 2 months, sweet cherries for up to 3 weeks, and strawberry up to 2 weeks, all carried out at 0°C. After cold storage, these commodities have up to 3-4 days of shelf life, which is usually at room temperature (around 20°C), on a shelf in the (super)market, for commercialization. During this period, is necessary to reduce the onset of decay development, to allow the consumer to take the fruit home and store it in a refrigerator for a few days without showing disease. During cold storage and shelf life, several countries do not allow the use of synthetic fungicides, or they have a restricted approved list of active ingredients, although for grapes it is also possible to use sulfur dioxide releasing pads during cold storage and transport. These releasing pads need to be removed when the temperature rises, to avoid phytotoxic effects on the berries in contact with the pads (Zoffoli *et al.* 2008). However, the use of sulphur dioxide is not allowed for organic table grapes (Mlikota Gabler and Smilanick 2001). Therefore, together with the consumer demand for food free of pesticide residues, the use of alternative means to control postharvest decay of such fruits has gained increasing interest (Elmer and Reglinski 2006; Lichter *et al.* 2006). Among these, the use of a coating has been widely investigated for

different crops, and chitosan was identified some decades ago as having the properties of an ideal coating for fruit and vegetables (Muzzarelli 1986). This natural biopolymer is obtained from chitin deacetylation (Muzzarelli and Muzzarelli 2003), and it can produce a film on treated surfaces, both with preharvest spraying and with postharvest application. Preharvest and postharvest chitosan treatment of table grapes, strawberries and sweet cherries reduces decay of these commodities when in storage, with dose-dependent effects seen. Chitosan usually treatment shows the best performance at the highest concentration used (often 1%, but in some trials also 2%, and occasionally 3%), while its effectiveness in decay control decreases with reduced dose (at 0.1% at times it has been shown to significantly reduce decay, although in other trials it has not been effective). Several chitosan-based commercial products are available on the market. Those include Chitogel (Ecobulle, France) (Ait Barka *et al.* 2004; Elmer and Reglinski 2006), Elexa (Safescience Inc., USA), Elexa 4 Plant Defense Booster (Plant Defense Booster Inc., USA) (Elmer and Reglinski 2006), Biochikol 020 PC (Gumitex, Lowics, Poland) (Patkowska 2005), Chito Plant (ChiPro GmbH, Bremen, Germany) (Romanazzi *et al.* 2007b), and Kendal Cops (Valagro, Atessa – CH, Italy, www.valagro.com). One of these, Chito Plant, was not different in its effectiveness in the control of postharvest decay of strawberries respect to chitosan dissolved in acetic acid (Romanazzi *et al.* 2007b). Commercial chitosan formulations have the advantage to show a lower viscosity with respect to the biopolymer dissolved in an acid solution, as also happens with the water soluble glycol chitosan (Romanazzi *et al.* 1999b, 2009).

## POSTHARVEST TREATMENTS

A large amount of data is available on the effectiveness of chitosan treatment applied to table grapes, strawberries, and sweet cherries after harvest. Single table grape berries wounded, treated with chitosan and later inoculated with *B. cinerea* showed a decrease in gray mold infections with respect to the control (Romanazzi *et al.* 2002). The best results were obtained with 1% chitosan, on berries kept 5 days at room temperature or stored 15 days at 0°C, and then exposed to 2 days shelf life. Similar results were obtained for small bunches dipped in chitosan solutions, artificially inoculated by spraying with a *B. cinerea* conidial suspension, and stored at cold (0°C) or room (20°C) temperatures. Treatment with chitosan at 0.5 and 1% decreased the spread of gray mold infection from a berry to the closest neighbours (nesting), both at room temperature and after cold storage (Romanazzi *et al.* 2002). The combined treatment of reduced doses of chitosan (0.1 and 0.5%) and ethanol (10 and 20%) provided an additive, and at times synergistic decay reduction of artificially inoculated gray mold for table grape berries kept 7 days at 15°C and bunches cold stored for 2 months, and then exposed to 3 days of shelf life (Romanazzi *et al.* 2007a). Also, the combination of 1% chitosan and a grapefruit seed extract improved decay control with respect to single applications in single berries, with and without the pedicel, stored 7 days at 25°C, and in bunches inoculated or not inoculated and stored for up to 4 weeks at 0°C (Xu *et al.* 2007). Postharvest chitosan application at different concentrations (1.5 and 2%) decreased *B. cinerea* infections in bunches artificially inoculated by wounding and kept at 25°C, while it was not effective when the biopolymer was applied before challenging with the pathogen (Camili *et al.* 2007). The effectiveness of chitosan to control gray mold infections in artificially inoculated berries and bunches changed greatly according to the acid used to dissolve the biopolymer, and acetic acid performed best (Romanazzi *et al.* 2009). Blue mold was reduced by chitosan dissolved in a list of acids, but not by malate and maleicate (Romanazzi *et al.* 2009). Treatments with 1 and 2.5% chitosan decreased antrachnose, which is caused by *Colletotrichum* sp., on artificially inoculated table grape berries kept 7 days at 24°C (Munoz *et al.* 2009).

Strawberries artificially inoculated with a conidial suspension of *B. cinerea*, and then dipped in 1 or 1.5% chitosan and stored for 21 days at 13°C showed a significant reduction in decay, as compared to the controls. The effectiveness of chitosan treatment was not different with respect to the commercial fungicide Rovral (a.i. iprodione), and no difference in decay control was seen between the two chitosan concentrations (El Ghaouth *et al.* 1991). Under similar conditions, when the biopolymer was applied to strawberries artificially inoculated with *R. stolonifer*, decay was decreased by more than 60%, as compared to the controls (El Ghaouth *et al.* 1992a). Chitosan coating was as also effective as the fungicide TBZ in controlling artificially inoculated gray mold and Rhizopus rot in strawberries held for up to 6 days at 13°C (Zhang and Quantick 1998). A water-soluble chitosan analogue, glycol chitosan, reduced artificially inoculated and naturally occurring gray mold infection on strawberries stored for 7 days at 3°C, followed by a 7-day shelf life (Romanazzi *et al.* 1999b). Strawberries dipped in 1% and 0.5% chitosan decreased gray mold infections from natural inoculum after a 10-day storage at 0°C, followed by 4 days shelf life (Romanazzi *et al.* 2000). *Cladosporium* sp. and *Rhizopus* sp. infections decreased in artificially inoculated strawberry fruit that were coated with chitosan and stored for up to 20 days at 4-6°C (Park *et al.* 2005). Postharvest treatment with chitosan dissolved in acetic, glutamic, hydrochloric and formic acid also decreased gray mold and Rhizopus rot infections after 3 days of storage at room temperature, and its effectiveness was not different from the chitosan-based commercial product Chito Plant (Romanazzi *et al.* 2007b).

Sweet cherries immersed for a few seconds in 1% chitosan solution and stored for 14 days at 0°C, followed by 7 days of shelf life, showed a significant reduction in brown rot and gray mold. An additive, and at times synergistic, decay reduction has been seen by combining chitosan and hypobaric treatment (Romanazzi *et al.* 2003). Reduced decay development has also been seen for sweet cherries treated with 1% chitosan and stored at 25 and at 2°C (Park and Zhao 2004).

## PREHARVEST APPLICATIONS

While there is relevant information about the effectiveness of postharvest chitosan treatments, fewer data are available concerning the evaluation of preharvest application in the control of postharvest decay of table grapes, strawberries and sweet cherries. On the other hand, this application is closer to the commercial scale, and is mostly suitable for fruit that have a bloom on the surface and/or can suffer postharvest wetting, such as table grapes and strawberries. The development of postharvest decay often arises from an inoculum that survives and accumulates on the fruit in the field and or in the packaging chain after the harvest. Table grape bunches sprayed in the field with chitosan at three different concentrations (1, 0.5 and 0.1%), either once, 21 days before harvest, or twice, 21 and 5 days before harvest, significantly reduced gray mold infections after 30 days storage at 0°C, followed by 4 days of shelf life (Romanazzi *et al.* 1999a). Decay control was not different with respect to grapes treated in the field with procymidone and stored with sulfur dioxide (Romanazzi *et al.* 2002). Berries with preharvest treatments with chitosan showed decreased incidence and severity of artificially inoculated postharvest gray mold, with the best results obtained 1-2 days after the application (Romanazzi *et al.* 2006). Blue mold was reduced by preharvest chitosan treatment or postharvest UV-C application, and their combination resulted in a synergistic reduction (Romanazzi *et al.* 2006).

In strawberries, *B. cinerea* infects the stamens, where it survives latently until the environmental conditions are proper for its spread, which usually occurs after harvest (Maas 1998). From the stamen residues, the pathogen infects the remaining parts of the fruit, and so basilar use of preharvest treatments is needed to reduce postharvest gray mold.



**Fig. 1** Sweet cherries preharvest treated with 1% chitosan (on the left box) and control (on the right), stored 2 weeks at 0°C, then exposed to 7 days shelf life.

Similarly, *Rhizopus* rot infections occur in the field, and mainly on fruit that are wet by direct rain, and they develop after the harvest, mainly with storage temperatures above 4°C. Strawberries sprayed with chitosan when the fruit were turning red and 10 days after, and then challenged with *B. cinerea* after the harvest and stored at 3 and 13°C showed a reduction in gray mold infections (Reddy *et al.* 2000). Strawberries treated with chitosan at full bloom, green fruit or whitening fruit showed a decrease in gray mold and *Rhizopus* rot infections from natural inocula after 10 days of storage at 0°C followed by 4 days of shelf life (Romanazzi *et al.* 2000). Decay control with 1% chitosan was in almost all treatments significantly better than the chemical standards, of procymidone at the full bloom and green fruit stage, and pyrimethanil at the whitening fruit stage. Spraying chitosan on strawberries three times (at the beginning of bloom, during full bloom, and at the end of bloom) resulted in a decrease in decay (Mazur and Waksmundzka 2001). Preharvest treatments with 1 and 2% chitosan decreased postharvest gray mold from natural inoculum, and after preharvest and postharvest inoculation these applications performed significantly better than a fungicide (Mazaro *et al.* 2008). The treatment with 1% chitosan also performed better than that with 2%, the latter being occasionally phytotoxic.

Little data are available on the control of postharvest decay of sweet cherries by preharvest chitosan treatments. Sweet cherries treated 7 days before the harvest with 0.1, 0.5 and 1% chitosan decreased gray mold and brown rot after 2 weeks of storage at 0°C followed by 7 days of shelf life, as compared to the untreated control (Romanazzi *et al.* 1999a) (**Fig. 1**). At the highest chitosan concentration, the decay reduction was not different with respect to that seen after application of tebuconazole. Finally here, preharvest chitosan and postharvest hypobaric treatments showed an additive effect in the control of total rots of sweet cherries in storage (Romanazzi *et al.* 2003).

## MECHANISMS OF ACTION

Chitosan has a double mechanism of action: it inhibits the development of the decay-causing fungi and it induces resistance responses in host tissues. The biopolymer has been reported to reduce germination and radial growth of a list of fungi that can cause decay on table grapes, strawberries and sweet cherries, such as *A. alternata* (El Ghaouth *et al.* 1992b; Romanazzi *et al.* 2001; Patkowska 2005; Abd-Allah and Hashem 2006), *B. cinerea* (El Ghaouth *et al.* 1992a, 1992b; Du *et al.* 1997; Romanazzi *et al.* 2001; Ben-Shalom *et al.* 2003; Ait Barka *et al.* 2004; Lira-Saldivar *et al.* 2006; Camili *et al.* 2007; Liu *et al.* 2007; Xu *et al.* 2007;

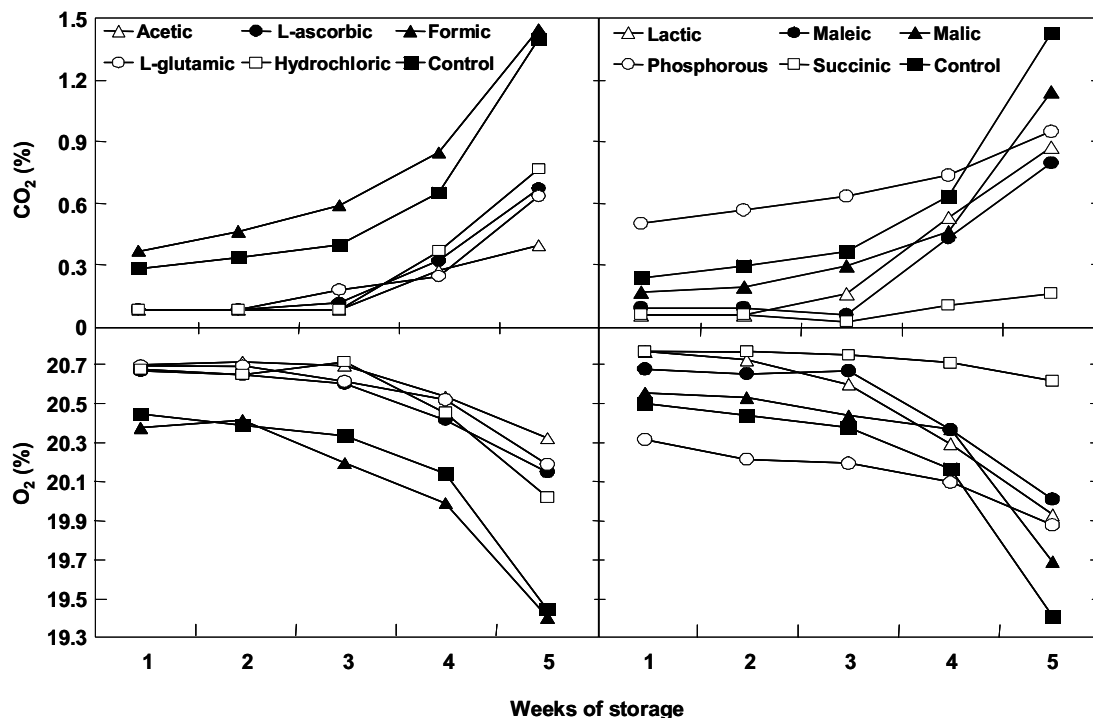
Badawy and Rabea 2009), *Cladosporium* sp. (Park *et al.* 2005; Sivakumar *et al.* 2005a; Abd-Allah and Hashem 2006), *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. in Penz. (El Ghaouth *et al.* 1992b; Bautista Banos *et al.* 2003; Sivakumar *et al.* 2005b; Ali and Mahmud 2008), *Colletotrichum* sp. (Munoz *et al.* 2009), *M. laxa* (Romanazzi *et al.* 2001), *P. expansum* (Sivakumar *et al.* 2005a; Liu *et al.* 2007; Yu *et al.* 2007), *R. stolonifer* (El Ghaouth *et al.* 1992a, 1992b; Velasquez-del Valle *et al.* 2008), and *Rhizopus* sp. (Park *et al.* 2005; Abd-Allah and Hashem 2006). A reduction in *B. cinerea* radial growth was also seen in Petri dishes with added 0.2% glycol chitosan (Romanazzi *et al.* 1999b).

Several physiological changes have also been observed in host tissues treated with chitosan. Table grape bunches with a preharvest spraying with chitosan showed a three-fold increase in phenylalanine ammonia-lyase (PAL) activity in the berry skin 24 h and 48 h after the application (Romanazzi *et al.* 2002). PAL elicitation was confirmed with table grapes sprayed in the vineyard and/or given a postharvest coating with chitosan, then stored at 0°C (Meng *et al.* 2008). Respiration of grapes dipped in 1% chitosan solutions decreases, as during storage of grapes at 0°C for 5 weeks, compared to the control, with different magnitudes according to the acid used to dissolve the biopolymer (Romanazzi *et al.* 2005). The greatest reduction in respiration was seen for dissolving the chitosan in acetic and succinic acids, and the former performed better in decay control (Romanazzi *et al.* 2009) (**Fig. 2**). A tendential reduction in respiration was seen for grape bunches treated with 0.5% chitosan, alone or combined with ethanol (Romanazzi *et al.* 2007a). The preharvest treatment with chitosan primed the elicitation of catechin and *trans*-resveratrol that occurs in the berry skin following UV-C treatment (Romanazzi *et al.* 2006). The treatment with 1% chitosan decreased hydrogen peroxide production in table grape berries, showing a protective and antioxidant effect (Romanazzi *et al.* 2007c). The hydrogen peroxide is involved in the first steps of the induction of resistance in the host cell by chitosan (Amorabé *et al.* 2009). Several physiological modifications have also been observed in table grapes exposed to preharvest and postharvest chitosan applications, with changes in soluble solid content, titrable acidity, total phenolic content, and enzyme activities, as superoxide dismutase, polyphenol oxidase, and peroxidase (Meng *et al.* 2008).

Chitosan-treated strawberries have shown a range of changes that are related to a slowed ripening, such as increased titratable acidity (El Ghaouth *et al.* 1991; Zhang and Quantick 1998; Reddy *et al.* 2000; Han *et al.* 2004; Chaiprasart *et al.* 2006; Hernandez-Munoz *et al.* 2006; Vargas *et al.* 2006; Mazaro *et al.* 2008), with delayed changes in pH (Han *et al.* 2004; Hernandez-Munoz *et al.* 2006; Vargas *et al.* 2006), antocyanin content (El Ghaouth *et al.* 1991; Zhang and Quantick 1998; Reddy *et al.* 2000; Vargas *et al.* 2006), soluble solids content (Chaiprasart *et al.* 2006; Vargas *et al.* 2006; Ribeiro *et al.* 2007), and with reduced ethylene production (Mazaro *et al.* 2008). Changes in enzyme activities in coated strawberries has been shown to involve chitinase, chitosanase and  $\beta$ -1,3-glucanase (El Ghaouth *et al.* 1992a; Zhang and Quantick 1998), and PAL, which increased three-fold in treated berries (Romanazzi *et al.* 2000). Moreover, a decreased respiration rate (El Ghaouth *et al.* 1991; Devlieghere *et al.* 2004; Vargas *et al.* 2006) and hydrogen peroxide production (Romanazzi *et al.* 2007b) have been shown in fruit treated with the biopolymer.

## CONCLUSIONS

Chitosan, an N-acetylated derivative of the polysaccharide chitin, is a biopolymer that has been under considerable investigation for applications in agriculture, biomedicine, and biotechnology, and in the food industry due to its biocompatibility, biodegradability, and bioactivity (Wu *et al.* 2005). Bautista-Banos *et al.* (2006) thoroughly discussed



**Fig. 2** Oxygen and carbon dioxide concentrations inside 0.5 liter volume plastic bags where Crimson Seedless grape clusters were enclosed after immersion in chitosan-acid solution stored at 0°C for 5 weeks. The 10 chitosan solutions were divided in two panels to avoid overlapping of lines, with control treatments (immersion in deionized water) in both panels. Reprinted from Romanazzi G, Mlikota Gabler F, Margosan DA, Mackey BE, Smilanick JL (2009) Effect of acid used to dissolve chitosan on its film forming properties and its ability to control postharvest gray mold of table grapes. *Phytopathology* 99, 1028-1036, with kind permission of APS, St. Paul, MN, USA.

the use of chitosan in the control of decay of fruit and vegetables. As there are relevant information on the direct inhibition of fungal pathogens, it is also well known the induced resistance in the host tissues (Choi *et al.* 2001; Repka 2001; Bi *et al.* 2007; Amborabé *et al.* 2009). No phytotoxic effects were observed after preharvest or postharvest chitosan applications on strawberries, sweet cherries and table grapes (El Ghaouth *et al.* 1991; Romanazzi *et al.* 2000, 2002, 2003; Camili *et al.* 2007), except when 2% was applied on strawberries (Mazaro *et al.* 2008) or when formic acid was used as chitosan solvent (Romanazzi *et al.* 2009). The chitosan coating did not induce any negative change in the sensorial properties of strawberries and table grapes (Devlieghere *et al.* 2004; Xu *et al.* 2007). In 2001, chitosan was introduced in the list of generally recognized as safe (GRAS) compounds by Food and Drug Administration (FDA) of USA (Harish Prashanth and Tharanathan 2007). The biopolymer is used as a common food adjuvant, it is safe for humans, and it is used in slimming diets, in tablets and in food processing (Shahidi *et al.* 1999). The use of edible coatings, either alone or together with modified atmosphere packaging, to maintain the properties and extend the storage of fresh-cut fruit and vegetables, was recently reviewed by Rojas-Graü *et al.* (2009). Chitosan has widespread application in the control of plant diseases of strawberry, including some that occur in the field but can have effects also after the harvest, such as *Phytophthora cactorum* (Lebert and Cohn) J. Schröt., which can affect the berries (Eikemo *et al.* 2000). The use of coating is promising also for sweet cherries (Martinez-Romero *et al.* 2006). The availability of chitosan commercial products, that can be easily dissolvable in water, provide a further alternative to the growers for the control of preharvest and postharvest diseases of strawberries, sweet cherries and table grapes. With its intrinsic properties, and because of the double activity on the host and on the pathogen, chitosan can be considered the first of a new class of plant protection products (Bautista-Banos *et al.* 2006). However, now that we have a lot of information about the effectiveness of chitosan, its application in large-scale tests and integration into commercial agricultural practices are key points that need to be

investigated further.

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