Food Control 41 (2014) 1–7

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Chitosan disrupts *Penicillium expansum* and controls postharvest blue mold of jujube fruit

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ARTICLE INFO

Article history:
Received 24 September 2013
Received in revised form
12 December 2013
Accepted 22 December 2013

Keywords:
Chitosan
Jujube fruit
Penicillium expansum
Postharvest disease
Plasma membrane

ABSTRACT

Chitosan has been shown to be effective for control of postharvest diseases on various fruit. However, little is known about the effect of chitosan on blue mold caused by *Penicillium expansum* on jujube fruit. Here we show that application of chitosan reduced disease development of blue mold caused by *P. expansum* in wounded and inoculated jujube fruit at 25 °C. Chitosan also provided an inhibitory effect on natural decay of jujube fruit during storage at 0 °C. Application of a chitosan coating to fruit had hardly any significant effect on the changes of weight loss, soluble solid contents, titratable acidity, and vitamin C, as storage time increased. To investigate the mechanisms underlying the effectiveness of chitosan against blue mold on jujube fruit, we analyzed the growth of *P. expansum* after chitosan treatment. Results indicated that spore germination, germ tube length and mycelial growth of *P. expansum* were significantly inhibited by chitosan in a concentration-dependent mode. Using the fluorescent probe propidium iodide, we found that the plasma membrane of *P. expansum* collapsed significantly after chitosan treatment. Further observation by electron microscopy revealed that plasma membrane of *P. expansum* was gradually disrupted after chitosan application. Our data suggest that chitosan may be potentially used for controlling postharvest diseases in jujube fruit without negative effect on fruit quality.

1. Introduction

Winter jujube (*Ziziphus jujube* Mill. cv. Dongzao) has flourished in China for over 4000 years (Li, Fan, Ding, & Ding, 2007). Due to its high nutritional value and savory taste, winter jujube has been extensively grown as a commercial crop. Although the fruit can be stored at low temperature for up to two months, they are very perishable and highly susceptible to postharvest color fading, browning, decay and water loss (Lin, Tian, Wan, Xu, & Yao, 2004). Spoilage fungi such as *Aspergillus flavus* and *Penicillium citrinum* can cause the production of mycotoxins and off-flavors, which in turn affect the quality and shorten shelf-life (Li, Xing, Jiang, Ding, & Li, 2009). *Alternaria* rot, caused by *Alternaria alternata*, is a major disease of jujube fruit in the field (Wang et al., 2009), but blue mold, caused by *Penicillium expansum*, is one of the most serious postharvest diseases of the fruit in China (Qin & Tian, 2004). Blue mold occurs during postharvest storage as well during shipping and marketing of the fruit, dramatically reducing shelf life and causing serious losses. Synthetic chemical fungicides are still the primary means of controlling postharvest diseases of jujube fruit in China but the adverse impact of synthetic chemical fungicides on human health and the environment is a cause for public concern. It is therefore necessary to develop alternatives to synthetic chemical control to reduce environment risks and raise consumer confidence (Zhang, Zheng, & Yu, 2007).

Chitosan is a deacetylated derivative of the linear polysaccharide chitin, which is the second most abundant polysaccharide found in nature. It consists of β-(1,4)-linked residues of −b-D-glucosamine(Cruz-Romero, Murphy, Morris, Cummins, & Kerry, 2013). The net positive charge of chitosan confers a variety of unique physiological and biological properties to this compound. It has broad-spectrum antibacterial activity (Jeon, Park, & Kim, 2001; Liu et al., 2006; Zheng & Zhu, 2003) and is also effective in inhibiting spore germination, germ tube elongation, mycelial growth, and sporulation of fungal phytopathogens (Liu, Tian, Meng, & Xu, 2007; Meng, Yang, Kennedy, & Tian, 2010; Palma-Guerrero, Jansson, Salinas, & Lopez-Llorca, 2008). The antimicrobial activity of chitosan varies according to its molecular weight, degree of deacetylation, pH of the chitosan solution, and the sensitivity of the

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http://dx.doi.org/10.1016/j.foodcont.2013.12.028

target organism (Xu, Zhao, Han, & Du, 2007). Kushwaha, Rai, and Singh (2010) has proposed that the antibacterial action of chitosan is brought about when positively charged chitosan reacts with negatively charged molecules at the cell surface, altering cell permeability and inhibiting the transport of compounds across the plasma membrane. However, the mechanism by which chitosan affects the development of phytopathogenic fungi has not been fully elucidated. As a biopolymer, chitosan, as well its derivatives, has excellent film forming properties (Fernández-Saiz, Sánchez, Soler, Lagaron, & Ocio, 2013) and is able to form a semi-permeable film on fruit, which might modify the internal atmosphere, as well as decrease weight loss due to transpiration and improve overall fruit quality (Jiang & Li, 2001; Zhang & Quantick, 1997). Chitosan coating could act as a mechanical barrier protecting fruits from pathogen infection and also as an exogenous elicitor of host-defense responses (Bautista-Baños et al., 2006; Meng, Li, Liu, & Tian, 2008; Sébastien, Stépahne, Copinet, & Coma, 2006), thus decreasing decay during storage.

Due to its multifunctional activity, chitosan has been proposed as a postharvest fungicide and preservative of fruit (No, Meyers, Prinyawiwatkul, & Xu, 2007; Zhang, Li, & Liu, 2011). Pre- and post-harvest application of chitosan has been reported to effectively control the decay of table grapes (Meng et al., 2008; Romanazzi, Nigro, Ippolito, Di Venere, & Salerno, 2002), and extend the shelf life of tomato (El Ghaouth, Ponnampalam, Castaigne, & Arul, 1992) and strawberry (El Ghaough, Arul, Ponnampalam, & Boulet, 1991). The effect of chitosan on control of blue mold and other types of decay in winter jujube fruit, as well as the effect on fruit quality has not been explored. The objectives of the present study were to investigate the effect of chitosan against blue mold caused by *P. expansum* and natural decay on jujube fruit and the detailed mechanisms involved. Influence of chitosan coating on fruit quality was also determined.

2. Materials and methods

2.1. Chitosan

Chitosan, with approximately a 90% deacetylation and average molecular weight of 350 kDa, was prepared at starting concentration of 25 g/L in 1% (v/v) HCl by stirring at 25 °C.

2.2. Pathogen inoculum

P. expansum was isolated from jujube fruit with typical blue mold symptoms and cultured on potato dextrose agar (PDA) at 25 °C. Spores of *P. expansum* were removed from 2-week-old cultures by adding 5 mL of sterile water containing 0.05% (v/v) Tween-80 to the Petri plates with gentle agitation. The spore suspensions were filtered through four layers of sterile cheesecloth to remove mycelia. Spore concentration was adjusted to the desired level with the aid of a hemocytometer prior to use.

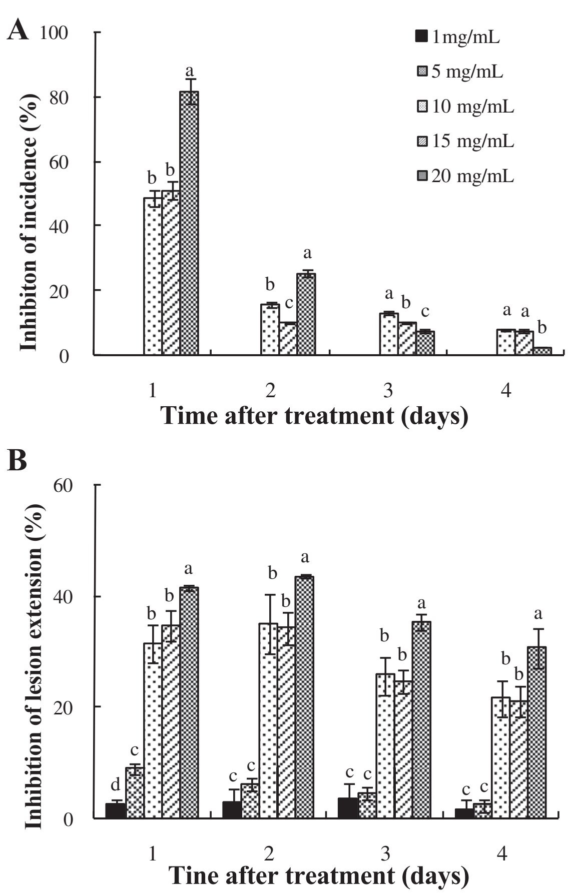

Fig. 1. Effect of chitosan on disease incidence (A) and lesion diameter (B) of artificially wounded jujube fruit inoculated with *P. expansum*. Bars represent standard deviations of the means based on three independent experiments. Within each day, columns followed by different letters are statistically different according to the Duncan's multiple range test ($P < 0.05$).

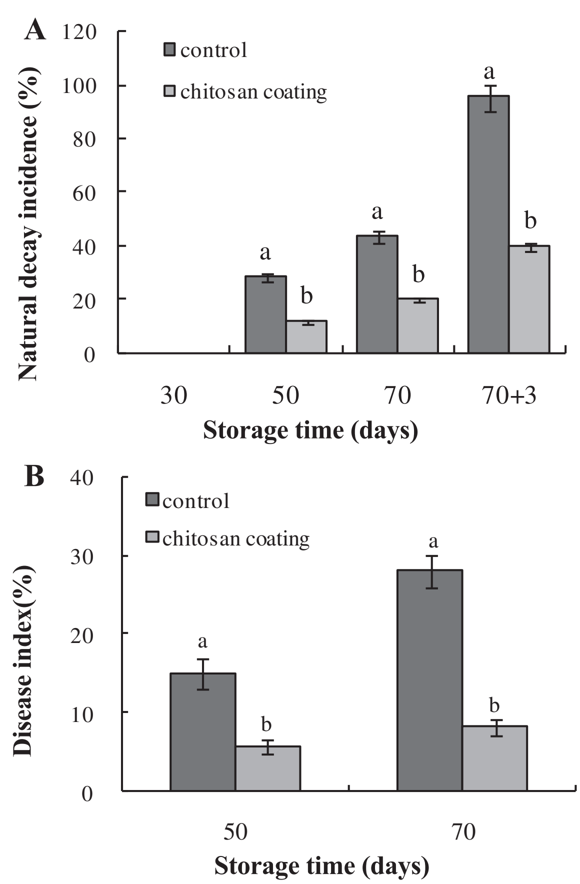

Fig. 2. Effect of chitosan coating on natural decay incidence (A) and disease index (B) in jujube fruit at different storage stage. Bars represent standard deviations of the means based on three independent experiments. Within each day, columns followed by different letters are statistically different according to the Duncan's multiple range test ($P < 0.05$).