

## Original Research Article

### Effect of chitosan biopolymer on microbial decay in tomato (*Solanum lycopersicum* L.)

#### Abstract

The current study sought to investigate the influence of chitosan biopolymer against microbial decay in tomato. In the present study, bulk-chitosan at different concentrations (0.01, 0.04, 0.08, 0.12, 0.16, 0.20% w/v) and Cu-CS NPs (0.01, 0.04, 0.08, 0.12, 0.16, 0.20% w/v) along with control were evaluated on tomato variety 'Dev' by dipping fruits for 6 min and stored at ambient temperature (27°C±2). Bulk-chitosan at 0.16% was found most effective to prevent microbial decay and maintain sensory evaluation from day 1 to days 21 as compared to control. Based on the aforementioned results and comparative evaluation of different doses of bulk-chitosan, we concluded that chitosan is very effective at less concentration and thus exert minimum chemical load on the treated tomatoes. Therefore, it may claim that chitosan biopolymer have potential to protects tomato against microbial decay.

**Keywords:** Chitosan, biopolymer, microbial decay, tomato

#### Introduction

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop after potato (FAO, 2021), and it is a model plant for researchers, especially for those studying the growth, yield and quality of fleshy fruits (Bertin and Genard, 2018). Tomato contains higher amounts of lycopene, a type of carotenoid with antioxidant properties which is beneficial in reducing the incidence of chronic diseases like cancer and many other cardiovascular disorders (Viskeli *et al.*, 2015). India is the 2<sup>nd</sup> largest (11.5%) producer of tomato in world. According to FAOSTAT (2021), tomato was grown world-wide in an area of 5.16 MH with the production of 189.13 MT and productivity of 36.6 MT per hectare. In India it was grown in 0.84 MH with the production of 21.18 MT and productivity of 25.06 MT per hectare. Chitosan is a linear amino polysaccharide of glucosamine and N-acetylglucosamine units and obtained by alkaline deacetylation of chitin extracted from the exoskeleton of

Comment [a1]: Paragraph starting distance

Comment [a2]: Paragraph starting distance

Comment [a3]: Bold

Comment [a4]: Bold

Comment [a5]: Bold

Comment [a6]: Bold

Comment [a7]: Paragraph starting distance

crustaceans such as shrimps and crabs as well from the cell walls of some fungi (Sucharitha *et al.*, 2018). It is being widely used in different fields ranging from medicine, tissue and bone engineering to food sector, cosmetics, textiles, pharmaceutical, biotechnology, paper industry and in waste-water treatment (Jiang and Li, 2005). In agriculture, chitosan is an ideal antimicrobial agent in preservation of fruits and vegetables. Chitosan is generally used to prevent microbial diseases and decay of fruits and vegetables through its pre- and post-harvest treatments (Bautista-Banos *et al.*, 2006). Its broad-spectrum antibacterial activity was first proposed by Allan. Chitosan is considered as a strong antimicrobial agent due to its positive surface charge which alters the microbial membrane structure that eventually leads to leakage of cellular fluids and death (Goyet *et al.*, 2009). The NH<sub>2</sub> group present in it is responsible for its bioactivity against microbial community through inhibition of gene expression and protein synthesis (Kong *et al.*, 2010). Chitosan has been used to prevent post-harvest losses in fruits and vegetables. It is also effective in reducing post-harvest diseases caused by various microbes like *Botrytis cinerea* (Elmer and Reglinski, 2006; Badawy and Rabea, 2009), *Penicillium expansum* (Liu *et al.*, 2007; Yu *et al.*, 2007), *Alternaria alternata* (Sanchez-Dominguez *et al.*, 2011), *Colletotrichum gloeosporioides* (Ramos-Garcia *et al.*, 2012) and *Rhizopus stolonifer* (Bautista-Banos *et al.*, 2006) by inhibiting spore germination, germ tube elongation and mycelial growth of fungal phytopathogens. Application of chitosan on tomato at 1.0, 1.5 and 2.0% concentrations significantly suppressed the development of *Rhizopus stolonifer* as compared with untreated fruit. In yet another study on tomato, it was found that application of chitosan at different concentrations in the range 0.01 to 1% significantly inhibited the growth of *Botrytis cinerea* and *Penicillium expansum* as compared with untreated tomato. Chitosan exhibits direct fungitoxic activity as well as elicits the biochemical defense responses in fruit (Liu *et al.*, 2007). On the basis of above facts, application of chitosan biopolymer was tested on tomato against microbial decay.

## Material and method

**Chitosan preparation:** In present study, bulk-chitosan (BCH) formulations were prepared by dissolving chitosan (Mol. Wt. 50,000–190,000 and 80% N-deacetylation; Sigma-Aldrich, St. Louis, MO, USA) into 1% glacial acetic acid to get final concentrations of 0.01, 0.04, 0.08, 0.12, 0.16 and 0.2% (w/v) with adjusting pH 5.1 with 1N NaOH. The chitosan formulations thus prepared were used to treat tomato fruits.

Comment [a8]: Bold

Comment [a9]: Bold

Comment [a10]: Paragraph section

Comment [a11]: Bold

Comment [a12]: Bold

Comment [a13]: Bold

Comment [a14]: Paragraph section

Comment [a15]: Bold

Comment [a16]: Bold

Comment [a17]: Bold

Comment [a18]: Bold

Comment [a19]: Bold

Comment [a20]: Bold

Comment [a21]: Reference is required for this section

**Experimental details:** Selected tomato fruits were dipped into different concentrations of bulk chitosan (0.01, 0.04, 0.08, 0.12, 0.16 and 0.20%, w/v in water) along with control (distilled water) for 6 min and stored at ambient temperature. Fungal decay was visually inspected in each treatment considering the extent of fungal mold on fruit surface in the scale ranging from 1 to 5 where 1 = normal (no decay on fruit surface), 2 = trace (up to 5% of fruit surface decayed), 3 = slight (5–20% of fruit surface decayed), 4 = moderate (20–50% of fruit surface decayed) and 5 = severe (>50% of fruit surface decayed). Results were expressed as fungal decay index. Overall acceptability of the samples was evaluated through the standard sensory evaluation techniques. The sensory attributes such as taste, flavor and acceptability was rated on a five point hedonic scale (9-Excellent, 7-Very good, 5-Good, 3-Fair, 1-Poor) by selected panel of judges (11 members).

Comment [a22]: Reference is required for this section

**Statistical analysis:** Statistical analysis was performed with JMP software version 12 (SAS, 2019) using Turkey Kramer HSD test. Each experiment was repeated twice wherein each treatment consisted of minimum three replicates having five tomatoes each.

Comment [a23]: Bold

## Result and discussion

In the present study, solutions of bulk-chitosan at different concentrations (0.01, 0.04, 0.08, 0.12, 0.16 and 0.20%, w/v) were prepared in 1% acetic acid. The pH of solutions was adjusted to 5.5 to eliminate the acidic damage to tomato fruits. Microbial decay was visually inspected up to 21 days considering the extent of microbial infection on fruit surface. It was recorded on 1 to 5 scales where 1 = normal (no decay on fruit surface), 2 = trace (up to 5% of fruit surface decayed), 3 = slight (5–20% of fruit surface decayed), 4 = moderate (20–50% of fruit surface decayed) and 5 = severe (>50% of fruit surface decayed). Decay rate increased with storage time due to microbial infection. The lowest decay (5% at scale of 2) was found in fruits treated with 0.08 and 0.16% bulk-chitosan (Table 1). Microbial decay contributes up to ~70% losses in tomato and is, therefore, very crucial to control it during storage. Bulk-chitosan at 2-4% concentrations considerably controlled gray mould cause by *Botrytis cinerea* in wound inoculated tomato fruits (Badawy and Rabea, 2009) whereas, at 0.5-1% concentrations significantly inhibited the growth of gray and blue moulds caused by *Botrytis cinerea* and *Penicillium expansum*, respectively in tomato fruits stored for 21 days at 2°C (Liu et al., 2007). Various concentrations of bulk chitosan considerably controlled decay of strawberry, pomegranate and table grapes during storage (Hajji et al., 2018; Candiret al., 2018; Felizianiet al., 2015; Gao et al., 2013). In our results, bulk chitosan at 0.08 and 0.16% concentrations significantly controlled tomato decay up to 21 days of storage (Table 1).

Comment [a24]: Paragraph starting distance

Comment [a25]: Paragraph section

Comment [a26]: Bold

Comment [a27]: Bold

Comment [a28]: Bold

Comment [a29]: Paragraph section

Results obtained in present investigation are better as compared with previous findings as very low concentration of bulk-chitosan comprehensively controlled the decay up to 21 days of storage at ambient temperature. Positively charged chitosan effectively degrades microbial cell wall and also boosts plant's immunity by enhancing defense enzymes activities (Bai *et al.*, 1988; Butler *et al.*, 1996). Therefore, chitosan is used to coat fruits and vegetables to control microbial infection during post-harvest storage. Sensory evaluation is another important parameter responsible for acceptability of tomatoes by consumers. Gao *et al.* (2013) studied that flavour of table grape significantly decreased after 15 days of storage in untreated fruits while with chitosan treatment, the sensory parameters were maintained up to the end of storage period. Our results showed that 0.16% bulk-chitosan was fairly effective to preserve color, texture, flavour and overall acceptability of tomato fruit (Table 2).

Comment [a30]: Bold

Comment [a31]: Bold

**Table 1: Decay at ambient temperature of tomato treated with different concentrations of BCH**

Treatment (%)	Decay			
	Day 0	Day 7	Day 14	Day 21
Control (Water)	0% (No decay)	20%(at scale of 3)	50%(at scale of 4)	>50% (at scale of 5)
Bulk-chitosan				
0.01	0% (No decay)	5%(at scale of 2)	20%(at scale of 3)	50% (at scale of 4)
0.04	0% (No decay)	5%(at scale of 2)	20%(at scale of 3)	>50% (at scale of 5)
0.08	0% (No decay)	0%(No decay)	0%(No decay)	5% (at scale of 2)
0.12	0% (No decay)	0%(No decay)	5%(at scale of 2)	20% (at scale of 3)
0.16	0% (No decay)	0%(No decay)	0%(No decay)	5% (at scale of 2)
0.20	0% (No decay)	5%(at scale of 2)	50%(at scale of 4)	>50% (at scale of 4)

**Table 2: Sensory evaluation at ambient temperature of tomato treated with different concentrations of BCH**

Treatment (%)	Sensory Evaluation			
	Day 0	Day 7	Day 14	Day 21
Control (Water)	9.0	7.0	3.0	1.0
Bulk-chitosan				

0.01	9.0	7.0	5.0	3.0
0.04	9.0	7.0	5.0	3.0
0.08	9.0	9.0	7.0	7.0
0.12	9.0	7.0	5.0	3.0
0.16	9.0	9.0	9.0	7.0
0.20	5.0	7.0	5.0	1.0

### Conclusion

The available review of literature and results of the present investigation concluded that amongst the various treatments, 0.16% bulk-chitosan significant results in microbial decay and sensory evaluation as compared with control up to 21 days of storage at ambient temperature ( $27\pm 2^{\circ}\text{C}$ ). Based on the aforementioned results and comparative evaluation of different doses of bulk-chitosan, we concluded that chitosan are very effective at less concentration and thus exert minimum chemical load on the treated tomatoes. Therefore, it is possible to assert that chitosan biopolymer has the capacity to shield tomatoes against microbial degradation.

Comment [a32]: Paragraph starting distance

### Reference

- Badawy, M.E.I. and Rabea, E.I. 2009. Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit. *Postharvest Biology Technology*, **51**: 110-117.
- Bai, R.K., Huang, M.Y. and Jiang, Y.Y. 1988. Selective permeabilities of chitosan-acetic complex membrane and chitosan-polymer complex for oxygen and carbon dioxide. *Polymer Bulletin*, **20**: 83-88.
- Bautista-Banos, S., Hernandez-Lauzardo, A.N., Velazquez-del Valle, M.G., Hernandez Lopez, M., Ait Barka, E., Bosquez-Molina, E. and Wilson, C.L. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection*, **25**: 108-118.
- Bertin, N. and Genard, M. 2018. Tomato quality as influenced by preharvest factors. *Scientia Horticulturae*, **233**: 264-276.

- Butler, B.L., Vergano, P.J., Testin, R.F., Bunn J.M. and Wiles J.L. 1996. Mechanical and barrier properties of edible chitosan films as affected by composition and storage. *Journal of Food Science*, **61**: 953-955.
- Candir, E., Ozdemir, A.E. and Aksoy, M.C. 2018. Effects of chitosan coating and modified atmosphere packaging on postharvest quality and bioactive compounds of pomegranate fruit cv. 'Hicaznar'. *Scientia Horticulturae*, **235**: 235-243.
- Dominguez, I., Federico, F., Fernando, P.R., Rafael, F. and Maria, I.G. 2012. Influence of preharvest application of fungicides on the postharvest quality of tomato (*Solanum lycopersicum* L.). *Postharvest Biology and Technology*, **72**: 1-10.
- Elmer, P.A.G. and Reglinski, T. 2006. Biosuppression of *Botrytis cinerea* in grapes. *Plant Pathology*, **55**: 155-177.
- Feliziani, E., Landi, L. and Romanazzi, G. 2015. Preharvest treatments with chitosan and other alternatives to conventional fungicides to control postharvest decay of strawberry. *Carbohydrate Polymers*, **132**: 111-117.
- Food and Agriculture Organization (FAO). 2021. FAOSTAT Statistical Database of the United Nation Food and Agriculture Organization (FAO) statistical division. Rome.
- Gao, P., Zhu, Z. and Zhang, P. 2013. Effects of chitosan-glucose complex coating on postharvest quality and shelf life of table grapes. *Carbohydrate Polymers*, **95**: 371-378.
- Goy, R.C., Britto, D. and Assis, O.B.G. 2009. A review of the antimicrobial activity of chitosan. *AssisPolimeros: Journal Ciencia E Tecnologia*, **19**(3): 241-247.
- Hajji, S., Younes, I., Affes, S., Boufi, S. and Nasri, M. 2018. Optimization of the formulation of chitosan edible coating supplemented with carotenoproteins and their use for extending strawberries postharvest life. *Food Hydrocolloids*, **83**: 375-392.
- Jiang Y.M. and Li Y.B. 2005. Effect of chitosan coating on postharvest life and quality of longan fruit. *Food Chemistry*, **73**: 139-143.
- Kong, M., Chen, X., Xing, K. and Park, H. 2010. Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal Food Microbiology*, **144**: 51-63.
- Liu, J., Tian, S., Meng, X. and Xu, Y. 2007. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biology and Technology*, **44**: 300-306.

- Ramos-Garcia, M., Bosquez-Molina, E., Hernandez-Romano, J., Zavala-Padilla, G., Terres Rojas, E., Alia-Tejacal, I., Barrera-Necha, L., Hernandez-Lopez, M. and Bautista-Banos, S. 2012. Use of chitosan-based edible coatings in combination with other natural compound, to control *Rhizopus stolonifer* and *Escherichia coli* DH5a in fresh tomatoes. *Crop Protection*, **38**: 1-6.
- SAS, 2019. JMP: User's Guide, Version 14.2. SAS Institute, Inc., Cary, NC, USA.
- Sucharitha, K.V., Beulah, A.M. and Ravikiran, K. 2018. Effect of chitosan coating on storage stability of tomatoes (*Lycopersicon esculentum* Mill). *International Food Research Journal*, **25**(1): 93-99.
- Viskelis, P., Radzevicius, A., Urbonaviciene, D., Viskelis J., Karkleliene R. and Bobinas C. 2015. Biochemical parameters in tomato fruits from different cultivars as functional foods for agricultural, industrial, and pharmaceutical uses. In: Plants for the future. H. ElShemy (Ed.), *InTech Open*, **10**: 5772-60873.
- Yu, T., Li, H.Y. and Zheng, X.D. 2007. Synergistic effect of chitosan and *Cryptococcus laurentii* on inhibition of *Penicillium expansum* infections. *International Journal of Food Microbiology*, **114**: 261-266.