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Research Article
Biochemical Effects of Chitosan Coating and Hot Water Dipping on Green Bean Decay During Cold Storage

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Abstract
Background and Objective: Normally fresh green bean (*Phaseolus vulgaris* L.) harvested seasonally and stored at room-temperature with short shelf-life (maximum 7 days). Therefore, the aim of this study was to determine the effect of chitosan coating (CC) and hot water dipping (HWD) on physico-chemical and sensory characteristics of the fresh-cut green bean and to determine extending storage periods and improve the quality. As well as to control color degradation and expand shelf-life of fresh-cut green bean. **Materials and Methods:** Fresh-cut green beans were treated with CC (at 0.5-1.5%) and HWD (at 45°C for 15 min or 55°C for 1 min). The treated samples were stored at 4°C in a refrigerator set at 85-90% relative humidity for 28 d. Physico-chemical properties (weight loss and decay, total soluble solids content, total acidity, chlorophyll and carotenoid contents, reducing sugars, protein, peroxidase and polyphenol oxidase activities, vitamin C and free phenolic compounds) and sensory properties were analyzed for all the treated samples at the storage points. **Results:** Fresh-cut green beans treated with CC at 1.5% exhibited the lowest decay (4.66%) while HWD treatments had the highest decay (25.5-48%) compared to 19.66% decay from the untreated control. Sensorial characteristics (color, skin loss, fibrousness, texture and flavor) were maintained and the quality of green bean pods improved under CC at 1.5%. Chitosan coating (1.5%) reduced weight loss, titratable acidity, peroxidase and polyphenol oxidase activities under the storage condition compared to other treatments. The reduction in peroxidase and polyphenol oxidase activities enhanced active oxygen-scavenging systems in the fresh-cut green bean. The phenolics and total soluble solids content increased with CC at 1.5% in fresh-cut green bean at 28 days of storage period. Also, protein and reducing sugars content increased when fresh-cut green bean was subjected to CC at 1.5%. **Conclusion:** These results showed that the quality of fresh-cut green bean could be amended and the shelf life extended by treating pods post harvest with CC at 1.5%.

Key words: Chitosan, green bean, hot water treatment, post-harvest decay

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Green bean (*Phaseolus vulgaris* L.) is one of the most nutritious leguminous that provide significant amounts of health benefiting vitamins, dietary fiber, proteins, carbohydrates as well as minerals [1]. Physiologically immature fleshy pods of this crop are harvested and consumed as a green vegetable. Green bean pods are subjected to continual biochemical and physiological changes predominantly higher respiration and transpiration after harvest, resulting in a comparatively short shelf-life even when stored at low temperatures. Consequently, expanding the shelf-life of the green bean is an essential factor for quality retention which can extend storability while also maintaining post-harvest quality. Several methods including chemical treatments such as calcium chloride, citric acid and ascorbic acid [2] and calcium lactate[3] have been used for commercial prolong vegetable preservation. Other methods such as ultraviolet irradiation[4], modified atmosphere packaging, anti-oxidant treatments and chlorine washing[5] are also commonly used to preserve fresh-cut vegetables.

Chitosan coating (CC), a natural product[6] and hot water dipping (HWD) have been developed as alternatives to chemical methods to expand the shelf-life and preserve the quality of fresh vegetables because of their excellent effectiveness in controlling growth of the pathogens, non-residue after treatment and little or no toxicity of using it[7]. Nevertheless, HWD can cause spoilage in green zucchini (*Cucurbita pepo* L.) and cucumber (*Cucumis sativus* L.)[8] and atypical softening in papaya (*Carica papaya* L.)[9]. Notwithstanding, green vegetables treated with HWD significantly reduced chilling injury[10-12]. Chitosan, a biodegradable, non-toxic polymer with antimicrobial activity and the ability to elicit defense responses in plant tissues has become a potential substitute treatment for preservation of fresh vegetables and fruits[13]. Chitosan coating has been used to extend storage periods and improve the quality of vegetables postharvest such as tomato and strawberry (*Fragaria*×*ananassa* Duch.)[14,15]. Significant increase and activity of β-1,3-glucanase, chitosanase and chitinase that control deterioration in fresh produce by preventing fungal growth has been reported in strawberries coated with chitosan[16].

Cutting of green bean at harvest has an enormous impact on quality attributed to accelerated physiological changes, including a higher respiration rate compared to the whole beans, limited shelf-life and rapid reduction in quality. Deterioration of color, an important quality attribute of fresh-cut green bean has been connected with some enzyme's activity such as peroxidase and lipoxygenase that stimulate undesirable changes in color[17]. Non-chemical treatments such as CC and HWD can potentially be used to prevent and mitigate quality losses and extend the shelf-life of the fresh-cut green bean. Therefore, this study aimed to assess the effect of HWD and CC on enzymatic (peroxidase and polyphenol oxidase) and non-enzymatic (carotenoid, vitamin C and free phenolic compounds) anti-oxidants to control color degradation and expand shelf-life, also enhance the nutritional properties of the fresh-cut green bean.

MATERIALS AND METHODS

Plant materials: Green bean, cultivar 'Royal Nel' was harvested from a local farm at Zagazig, Sharkia, Egypt in May, 2018 based on commercial maturity indexes such as shape, firmness and color. Green bean pods were harvested in uniform size and shape (~14 cm in length and 6 mm in diameter). Immediately after harvesting, the pods were transported to the laboratory at the Biochemistry Department, Faculty of Agriculture, Zagazig University, Egypt.

Treatments and storage conditions: Green bean pods were washed using distilled water and kept for air dried. Then the tips and spoiled areas of green bean pods were removed. Afterward, the green bean pods were homogenized into 10 cm long pieces and exposed to two treatments with two levels each, hot water dipping (at 45°C for 15 min or 55°C for 1 min) and Chitosan coating (at 0.5 or 1.5%). The chitosan (Sigma-Aldrich, Seelze, Germany) was prepared by dissolving the appropriate amount of chitosan in acetic acid (0.5% v/v) and 0.5% (v/v) Tween-80 (Sigma-Aldrich, Seelze, Germany) to obtain the working solution. An equal amount of the pods was dipped into 0.5-1.5% chitosan solution for 1 min to allow the chitosan to coat the whole surface of the pods. An untreated amount of the green bean pods was included as a control. After treatments, the pods were divided into groups 150 g each, packed in foam plates, put in polyethylene pages and then stored in a cold room set at 4°C under 85-90% relative humidity. The plates were set up as a randomized complete block design with six replications of each treatment and each plate represented a block.

Physico-chemical and sensory properties

Weight loss and decay: Green bean pods weight losses (expressed as a percentage) were evaluated every 7 for 28 days. The decay percentage was estimated at the end of the storage period (28 days) as the total number of pods displaying decay symptoms (rotting) for each treatment.

Sensory evaluation: Five sensory characteristics (color, texture, fibrousness, skin loss and flavor) of fresh-cut green beans were evaluated according to Aparicio-Cuesta *et al.* [18]. A member panel trained for fresh-cut green bean evaluation was assigned to score each characteristic using a descriptive method based on 5-point category scales adapted for green bean. The extreme values of the sensory descriptors were based on a scale 1 to 5: fibrousness (1, non-fibrous; 5, extremely fibrous), color (l: very light green, 5: very dark green), flavor (l: very weak green bean flavor, 5: very strong green bean flavor), texture (l: very soft, 5: very hard) and loss of skin (l: no skin loss, 5: marked loss of skin).

Total soluble solids (TSS) content and total acidity (TA): Ten grams of chopped green bean were homogenized with 80 mL of double distilled water and filtered through a filter paper. The filtrate was centrifuged for 1 min at 3000 rpm. The TSS was measured in the supernatant using a refractometer (Palette PR-101, Tokyo, Japan).

Titratable acidity was measured by titration method as described by AOAC[19]. The TA was expressed as percent of citric acid according to AOAC[19].

Determination of chlorophyll and carotenoid: Total chlorophyll and carotenoid contents were estimated at 645, 663 and 470 nm, respectively using spectrophotometer (UV/VIS spectrometer, T80)[20]. The data were calculated and presented as mg /100 g fresh weight.

Determination of reducing sugars: Reducing sugars were determined following the method of Guo *et al.*[21] using 3,5-dinitrosalicylic acid reagents and measured spectrophotometrically (UV-VIS spectrometer, T80) at 540 nm. To quantify the reducing sugars, a standard curve using serial dilutions of standard glucose solutions was prepared.

Determination of protein: Total protein was estimated according to the method of Bradford[22]. The data were calculated and presented as mg g^{-1} fresh weight.

Determination of peroxidase and polyphenol oxidase activities: Fresh pods (200 mg) were homogenized in 1 mL of 0.1 M phosphate buffer (pH 6.5) and centrifuged at 3000 rpm for 1 min. The enzyme's activity was determined in the supernatant according to the method described by Urbanek *et al.*[23]. For peroxidase activity, the following reaction mixture was prepared, 0.2 mL enzyme extract, 3.5 mL 0.1 M phosphate buffer (pH 6.5), 0.3 mL 0.1% o-dianisidine solution and 0.2 mL 0.2 M hydrogen peroxide. To measure the enzyme activity, the mixture was incubated for 10 min at 30°C and then o-dianisidine oxidation was determined by changes in optical density absorbance at 430 nm using a UV-Visible spectrometer. The peroxidase activity in the soluble extract was expressed as unit[23] mg protein min^{-1}. Polyphenol oxidase activity was quantified according to Kasim and Kasim[2]. To measure the enzyme activity, the reaction mixture was incubated for 5 min at 25°C and then catechol oxidation was determined by changes in optical density absorbance at 410 nm using a UV-Visible spectrometer. The polyphenol oxidase activity in the soluble was expressed as unit mg protein min^{-1}.

Vitamin C determination: Vitamin C was determined as mg/100 g fresh weight in the green bean pods following the method described by Pearson[24].

Free phenolic compounds determination: Free phenolic compounds were measured as described by AOAC[19] using folin-ciocalteu reagent. The results were expressed as mg g^{-1} fresh weight.

Statistical analysis: All data were subjected to ANOVA (two-way test) using the MSTAT-C statistical package[25]. Different letters in the tabulated data or above the bars in the figured data indicate significant differences by Fisher's Protected LSD test at ($p<0.05$).

RESULTS

Decay (%) and sensory evaluation: There were significant differences among the treatments in percentage of fresh-cut green bean decay and sensory evaluation at 28 days after treatment (Table 1). The fresh-cut green bean treated HWD at 55°C for 1 min recorded the highest pod decay (48%), while the fresh-cut green bean treated with 1.5% CC recorded the lowest decay (4.66%) among all treatments. High discoloration rates were observed in green bean pods treated with HWD either 55°C for 1 min or 45°C for 15 min. The lowest rate of pod discoloration was observed in green bean treated with 1.5% CC. Also, there were significant differences among the treatments concerning skin loss, texture and flavor (Table 1). The fresh-cut green bean treated with CC at 1.5% resulted in the lowest degradation about skin loss and fibrousness in addition to having the highest texture and flavor compared to the control and other treatments.

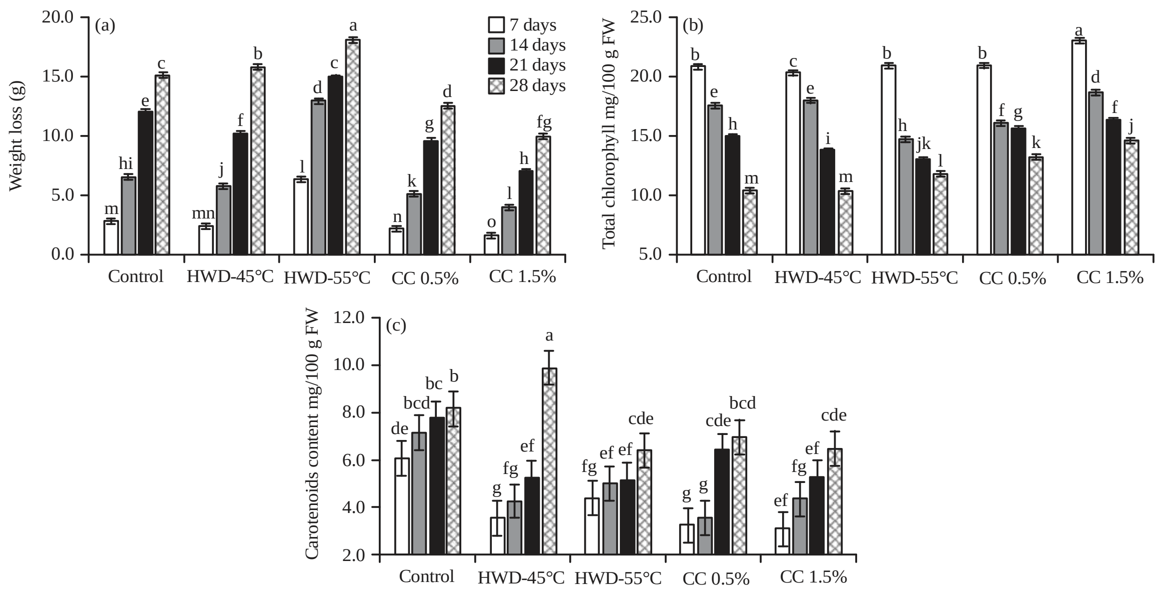

Fig. 1(a-c): (a) Percentage weight loss, (b) Total chlorophyll content and (c) Carotenoid content of fresh-cut green bean stored at 4°C in response to different treatments

Different letters above the bars indicate significant differences by Fisher's test ($p<0.05$)

Table 1: Sensory evaluation of fresh-cut green beans under different treatments at the end of storage

Treatments	Decay (%)	Color	Skin loss	Fibrousness	Texture	Flavor
Control	19.66^{c}	3.20^{b}	1.90^{c}	1.70^{c}	2.66^{b}	2.10^{c}
HWD (45°C, 15 min)	25.50^{b}	2.63^{d}	2.60^{b}	2.13^{b}	2.06^{c}	1.86^{c}
HWD (55°C, 1 min)	48.00^{a}	2.10^{e}	3.23^{a}	3.13^{a}	1.63^{d}	1.36^{d}
Chitosan 0.5 (%)	12.33^{d}	2.93^{c}	1.93^{c}	1.46^{cd}	2.63^{b}	2.56^{b}
Chitosan 1.5 (%)	4.66^{e}	3.53^{a}	1.33^{d}	1.10^{d}	3.50^{a}	3.33^{a}
LSD (0.05)	3.11	0.24	0.41	0.37	0.31	0.40

Means with the same letter in a column are not significantly different at $p<0.05$

Weight loss of green bean: Figure 1a demonstrated that fresh-cut green bean pod weight loss was increased with time during the 28-d storage period for all treatments, however, the highest weight loss was recorded in the HWD treatment at 55°C (18%), while the lowest weight loss was recorded in the CC at 1.5% treatment (10%) at the end of the storage period.

Total chlorophyll and carotenoids: Total chlorophyll of fresh-cut green bean pods was decreased as storage time increased for all treatments during the 28 days period (Fig. 1b). After 28 days storage, the untreated fresh-cut green bean (control) and the treated with HWD at 45°C for 15 min had the lowest total chlorophyll content compared to other treatments, while the highest content of total chlorophyll was recorded in the fresh-cut green bean treated with CC at 1.5% after 7 days of storage. Carotenoid content of fresh-cut green bean pods increased as storage period increased over 28 days (Fig. 1c). By the end of the storage period, the fresh-cut green bean treated with HWD at 45°C for 15 min resulted in the highest carotenoid content, while the lowest carotenoid content was observed in the fresh-cut green bean treated with HWD (55°C for 1 min) followed by the fresh-cut green bean treated with CC at 1.5%.

Total soluble solids, protein content, reducing sugars and total acidity: Total soluble solids in fresh-cut green bean pods increased continuously during the 28 days storage period for all treatments (Fig. 2a). The fresh-cut green bean pods treated with CC at 1.5% recorded the highest amount of TSS, while the lowest amount was recorded in the untreated fresh-cut green bean pods (control) at the end of the storage period. Protein content in fresh-cut green bean significantly increased in the green bean pods treated with CC at 1.5% compared to other treatments (Fig. 2b). The content of reducing sugars in fresh-cut green bean decreased with the progress of the storage period for all treatments (Fig. 2c). The fresh-cut green bean pods treated with CC at 1.5% recorded the highest amount of reducing sugars after 7 days of storage. At the end

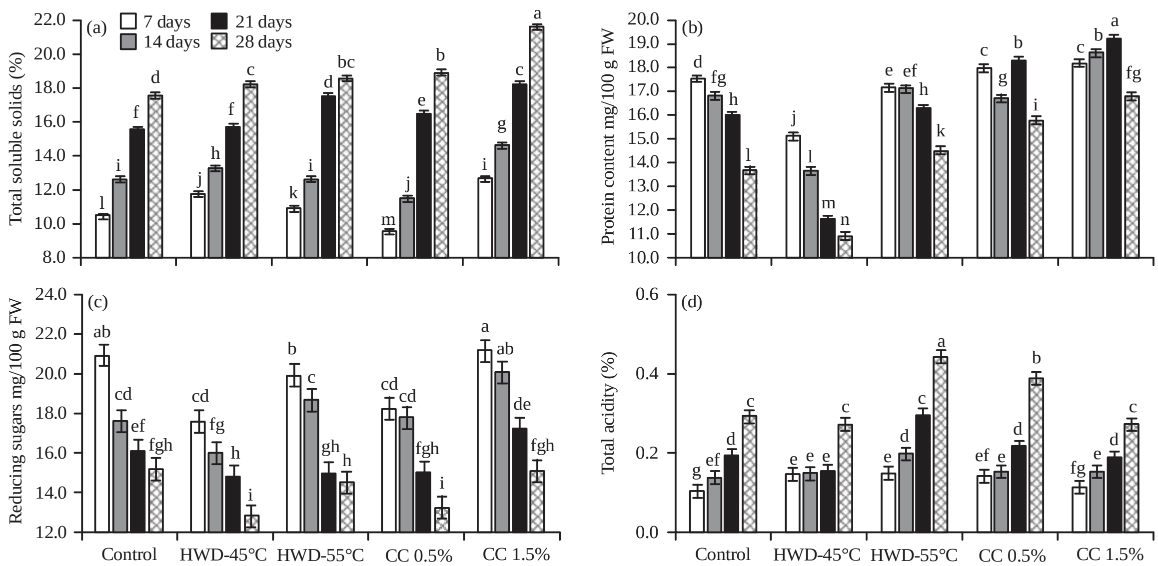

Fig. 2(a-d): (a) Percentage of total soluble solids, (b) Protein content, (c) Reducing sugars content and (d) Percentage of total acidity of fresh-cut green bean stored at 4°C in response to different treatments

Different letters above the bars indicate significant differences by Fisher's test ($p<0.05$)

of the storage period (28 days), there were not significantly different in the reducing sugars content among all the treatments. Total acidity was recorded the highest value in the fresh-cut green bean treated with HWD at 55°C for 1 min and CC treatment at 0.5% after 28 days of storage (Fig. 2d).

Vitamin C and phenolic contents: Vitamin C content increased in fresh-cut green bean treated with CC at 1.5% during the first 21 days of storage compared to other the treatments, with the highest amount observed at 21 days of storage (Fig. 3a). At the end of storage, vitamin C content decreased in the all treatments as storage duration increased, however, the fresh-cut green bean pods treated with CC at 1.5% was still significantly higher than other treatments at the same timing. At the same time, the lowest amount vitamin C was observed in the fresh-cut green bean pods treated with HWD at 55°C for 1 min. As the duration of the storage increased, the phenolic content of fresh-cut green bean increased for all treatments (Fig. 3b). At the end of the storage period, the highest level of phenolics was recorded in the fresh-cut green bean pods treated with CC at 1.5%, followed by the green bean pods treated with HWD at 45°C for 15 min.

Polyphenol oxidase and peroxidase activity: By increasing the storage period, the action of polyphenol oxidase in the fresh-cut green bean increased for the untreated control and HWD treatment at 45°C for 15 min (Fig. 3c). The fresh-cut green bean pods treated with CC at 1.5% significantly inhibited polyphenol oxidase activity. Although polyphenol oxidase activity increased with time for green bean pods treated with CC at 0.5% and HWD at 55°C for 1 min, the contents were significantly lower than the untreated control particularly after 28 d of storage.

Peroxidase activity of fresh-cut green bean was highest for the untreated control followed by the green bean treated with HWD at 55°C for 1 min after 7 days of the storage and increased afterward until the end of storage at 28 days (Fig. 3d). The level of peroxidase activity increased correspondingly for the 1.5% CC treatment. The untreated fresh-cut green bean (control) recorded the maximum activity of peroxidase at the end of storage, while the minimum activity was observed in the fresh-cut green bean pods treated with CC 1.5% after 7 days of storage.

DISCUSSION

Fresh vegetables and fruits shelf-life and quality are highly related to the biochemical processes that take place during post-harvest and storage. Green bean has a high respiration rate and heat emission, which have an impact on the metabolic processes and stimulate acceleration of decay, thereby limiting the shelf-life of the green bean after harvest to a maximum period of 0-4 weeks depending on post-harvest treatment[26]. Therefore, to enhance storability and quality of this unpreserved fresh produce, the use of

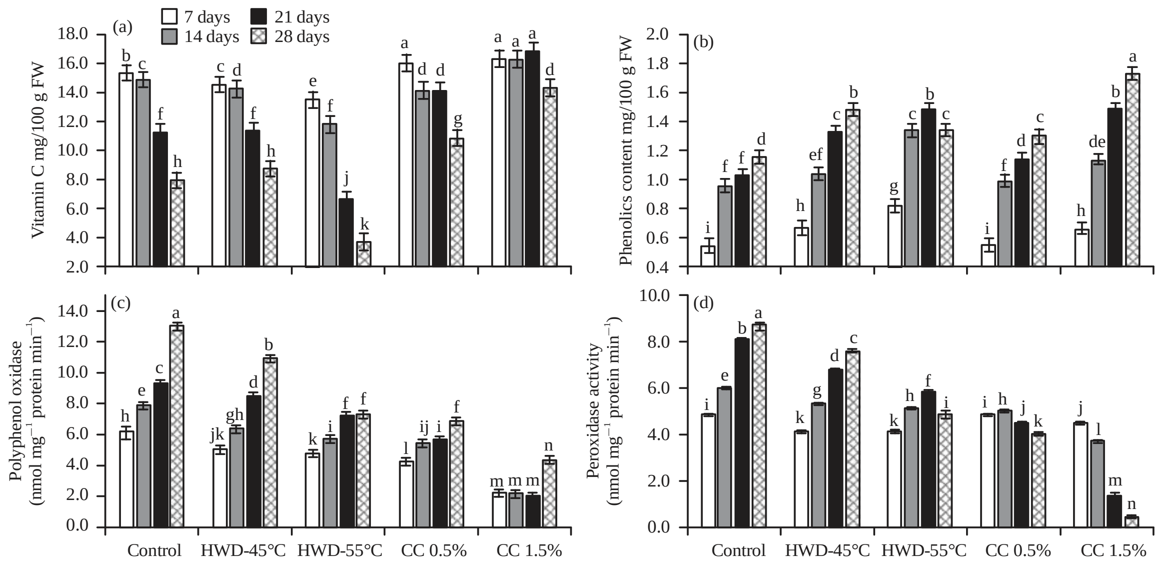

Fig. 3(a-d): (a) Vitamin C content, (b) Content of free phenolics, (c) Polyphenol oxidase and (d) Peroxidase activities of fresh-cut green bean stored at 4°C in response to different treatments

Different letters above the bars indicate significant differences by Fisher's test ($p<0.05$)

semi-permeable coatings has been proposed[14]. In the current study, applying chitosan coating (CC) to the fresh-cut green bean pods was generally more effective in improving their storability when stored at 4°C compared to the hot water dipping (HWD) treatment and the untreated control. Improvement of storability and quality of the fresh-cut green bean pods treated with CC could be attributed to the ability of CC to make a semi-permeable film around the green bean pods[27], which controlled the internal temperature of the pods as well as reduced transpiration losses. Treatment of fresh-cut green bean pods with CC at 1.5% preserved peroxidase and polyphenol oxidase activities at steady levels resulting in regular production of free radicals that improved quality and storability of the green bean pods. Moreover, Zhang *et al.*[6] reported that total polyphenols were increased when treating several fruits with chitosan and stimulate the key enzyme in the phenol synthesis pathway including phenylalanine ammonia lyase. There were significantly differed among the treatments in the green been pods decay ($p<0.05$). The fresh-cut green bean pods treated with HWD at 55°C for 1 min recorded the highest percentage of pod decay (48%) which associated with a high activity of peroxidase and polyphenol oxidase compared to the other treatments. Fresh-cut green bean pods treated with 1.5% of CC preserved color and prevented chlorophyll degradation during storage (Fig. 1b). Because chlorophyll degradation is a major challenge for food processors, many studies have been conducted to determine ways to mitigate or minimize degradation in order to produce high-quality fresh vegetable products. One of the indexes of decreased quality in the fresh-cut green bean is a loss of chlorophyll content. In contrast, significant differences in carotenoid content ($p<0.05$) of fresh-cut green bean pods were observed with storage time in the present study. This result agrees with Bouvier *et al.*[28], who reported an increase in the carotenoid content in pea (*Pisum sativum* L.) pods during cold storage and attributed that to the anti-oxidant properties in scavenging ROSs. In this regard, using CC to treat fresh-cut green bean pods before storage could limit the formation of ROSs and/or motivate some other mechanisms for quenching the generated ROSs in the stored pods. Furthermore, Kendra and Hadwiger[29] reported that chitosan application on pea pods induced the production of phytoalexin Pisatin in the pods which have antifungal activity against a wide range of fungi. Chitosan coating at 1.5% showed significantly different ($p<0.05$) regards to the highest number of phenolic compounds in fresh-cut green bean pods at the end of storage (Fig. 3b). The effect of chitosan in increasing phenolic compounds due to the accumulation of hydrogen peroxide (H_2O_2) in treated material, which stimulate a hypersensitive reaction as a result of phenolic compound deposition and oxidative microburst[30]. Treating the green pods with CC resulted in low level of titratable acid due to its effect minimizing the transpiration rate and preserving water in pods[6]. Furthermore, CC application on the green bean increased the total phenolic and flavonoid contents, while decreased ascorbic acid and carotenoid contents after cold storage (5±1°C)[31]. Also, the present results showed decreasing of vitamin C levels during the storage period in the untreated control and HWD treatments while CC treatment enhanced vitamin C content (Fig. 3a) ($p<0.05$). The reduction in ascorbic acid content may be due to the increase in ascorbic acid oxidase activity in the fruits and vegetables that convert the ascorbic acid dehydroascorbic acid. Polyphenol oxidase is known as an oxidative enzyme that catalyzes the oxidation reaction of phenolic substrates and also accountable for enzymatic browning[32]. In the current study, polyphenol oxidase activity of fresh-cut green bean was found to be high in the untreated control and significantly different ($p<0.05$) in the 1.5% CC treatment (Fig.3c). This observation was probably attributed to the inhibition effect of chitosan coating at 1.5% on polyphenol oxidase activity. Ramzani *et al.*[13] reported that chitosan coating is frequently used in food preservation as an inhibitor of browning reaction enzymes and an antioxidant agent. Treatment of green bean pods with CC limited symptoms of chilling and decay because of its ability to maintain fibrousness, texture and enhance total chlorophyll content. Treating strawberries with chitosan effectively maintained its sensorial properties and prolonged the postharvest shelf-life[14]. In contrast, when the fresh-cut green bean pods treated with HWD or untreated, it had high skin loss and chilling injury. In the present study, treating fresh-cut green bean pods with CC 1.5% resulted in the minimum weight loss and consequently the least percentage of rubbery appearance ($p<0.05$). Previous study showed that treating fruits with chitosan has a positive effect in reducing respiration and weight loss[6]. During storage of vegetable and fruits, low respiration rates can slow down sugar losses, which can affect the shelf-life and storage quality of vegetable and fruits. Overall, there was a reduction in reducing sugars for all treatments at the end of the storage period ($p<0.05$). Pariasca *et al.*[33] attributed this reduction in sugars to the normal senescence processes, which persist during the storage period. Furthermore, Martins *et al.*[34] reported that starch conversion to reducing sugars could prevent green beans from chilling injury under low temperature storage.

CONCLUSION

Chitosan coating treatment at 1.5% was effective in retaining the color of fresh-cut green bean during storage at 4°C. Similarly, fresh-cut green bean treated with CC (1.5%) significantly reduced titratable acidity, weight loss, pod decay and chilling injury compared to the other treatments. However, the activity of polyphenol oxidase in green bean pods was reduced when treated with high level of CC (1.5%).

SIGNIFICANCE STATEMENT

This study discovered that the chitosan coating at concentration 1.5% can be beneficial for enhancing the sensorial characteristics and maintained the quality of green bean pods during the storage. This study will help the researchers and farmers to uncover the critical areas of postharvest of green bean that many researchers were not able to explore. Thus, a new theory on extending shelf-life of fresh-cut green bean may be arrived at.

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REFERENCES

1. Brigide, P., S.G. Canniatt-Brazaca and M.O. Silva, 2014. Nutritional characteristics of biofortified common beans. Food Sci. Technol., 34: 493-500.
2. Kasim, R. and M.U. Kasim, 2015. Biochemical changes and color properties of fresh-cut green bean (*Phaseolus vulgaris* L. cv. gina) treated with calcium chloride during storage. Food Sci. Technol., 35: 266-272.
3. Rico, D., A.B. Martin-Diana, J.M. Frias, J.M. Barat, G.T.M. Henehan and C. Barry-Ryan, 2007. Improvement in texture using calcium lactate and heat-shock treatments for stored ready-to-eat carrots. J. Food Eng., 79: 1196-1206.
4. Kasim, M.U. and R. Kasim, 2008. Double-sided UV-C treatments delayed chlorophyll degradation but increased chilling injury of green bean (*Phaseolus vulgaris* L. cv. Helda) during storage. J. Food Agric. Environ., 6: 176-180.
5. Ahn, H.J., J.H. Kim, J.K. Kim, D.H. Kim, H.S. Yook and M.W. Byun, 2005. Combined effects of irradiation and modified atmosphere packaging on minimally processed Chinese cabbage (*Brassica rapa* L.). Food Chem., 89: 589-597.
6. Zhang, H., R. Li and W. Liu, 2011. Effects of chitin and its derivative chitosan on postharvest decay of fruits: A review. Int. J. Mol. Sci., 12: 917-934.
7. Barkai-Golan, R. and D.J. Phillips, 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. Plant Dis., 75: 1085-1089.
8. Jacobi, K.K., L.S. Wong and J.E. Giles, 1996. Postharvest quality of zucchini (*Cucurbita pepo* L.) following high humidity hot air disinfestation treatments and cool storage. Postharvest Biol. Technol., 7: 309-316.

9. Paull, R.E., 1995. Preharvest factors and the heat sensitivity of field-grown ripening papaya fruit. Postharvest Biol. Technol., 6: 167-175.
10. El-Hamahmy, M.A., A.I. ElSayed and D.C. Odero, 2017. Physiological effects of hot water dipping, chitosan coating and gibberellic acid on shelf-life and quality assurance of sugar snap peas (*Pisum sativum* L. var. *macrocarpon*). Food Packaging Shelf Life, 11: 58-66.
11. Mencarelli, F., B. Ceccantoni, A. Bolini and G. Anelli, 1993. Influence of heat treatment on the physiological response of sweet pepper kept at chilling temperature. Acta Hortic., 343: 238-243.
12. Sabehat, A., D. Weiss and S. Lurie, 1996. The correlation between heat-shock protein accumulation and persistence and chilling tolerance in tomato fruit. Plant Physiol., 110: 531-537.
13. Ramzani, P.M.A., M.S. Coyne, S. Anjum, W.U.D. Khan and M. Iqbal, 2017. *In situ* immobilization of Cd by organic amendments and their effect on antioxidant enzyme defense mechanism in mung bean (*Vigna radiata* L.) seedlings. Plant Physiol. Biochem., 118: 561-570.
14. El Ghaouth, A., R. Ponnampalam, F. Castaigne and J. Arul, 1992. Chitosan coating to extend the storage life of tomatoes. HortScience, 27: 1016-1018.
15. Ribeiro, C., A.A. Vicente, J.A. Teixeira and C. Miranda, 2007. Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biol. Technol., 44: 63-70.
16. Zhang, D.L. and P.C. Quantick, 1998. Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. J. Hort. Sci. Biotechnol., 73: 763-767.
17. Cano, M.P., A. Hernandez and B. de Ancos, 1997. High pressure and temperature effects on enzyme inactivation in strawberry and orange products. J. Food Sci., 62: 85-88.
18. Aparicio-Cuesta, M.P., M.P. Mateos-Notario and J.C. Rivas-Gonzalo, 1992. Sensory evaluations and changes in peroxidase activity during storage of frozen green beans. J. Food Sci., 57: 1129-1143.
19. AOAC., 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., Pages: 684.
20. Litehtenhaler, H.K. and A.R. Wellburn, 1983. Determination of total crotenoids and chlorophylls a and b of leaf extracts in different solvents. Proceedings of the Biochemistry Society Transection 603rd Meeting, (BSTM'83), Liverpool, UK., pp: 591-592.
21. Guo, L., Y. Ma, D.W. Sun and P. Wang, 2008. Effects of controlled freezing-point storage at 0°C on quality of green bean as compared with cold and room-temperature storages. J. Food Eng., 86: 25-29.
22. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
23. Urbanek, H., E. Kuzniak-Gebarowska and K. Herka, 1991. Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Physiol. Plant., 13: 43-50.
24. Pearson, D., 1970. The Chemical Analysis of Foods. 6th Edn., T.A Constable Ltd., London.
25. Nissen, O., 1990. Mstat-C: A microcomputer program for the design management and analysis of agronomic research experiments. Crop and Soil Sciences Department, Michigan State University, East Lansing, Michigan, USA.
26. Sanchez-Mata, M.C., M. Camara and C. Diez-Marques, 2003. Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: Macronutrients. Food Chem., 80: 309-315.
27. Bai, R.K., M.Y. Huang and Y.Y. Jiang, 1988. Selective permeabilities of chitosan-acetic acid complex membrane and chitosan-polymer complex membranes for oxygen and carbon dioxide. Polym. Bull., 20: 83-88.
28. Bouvier, F., R.A. Backhaus and B. Camara, 1998. Induction and control of chromoplast-specific carotenoid genes by oxidative stress. J. Biol. Chem., 273: 30651-30659.
29. Kendra, D.F. and L.A. Hadwiger, 1984. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. Exp. Mycol., 8: 276-281.
30. Han, H. and B.K. Baik, 2008. Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*) and their quantitative changes during processing. Int. J. Food Sci. Technol., 43: 1971-1978.
31. El-Malak, G., 2007. Evaluation of free radical scavenging by natural antioxidants in some fresh, blanched, cold and frozen storage of vegetables. Ann. Agric. Sci., 52: 367-374.
32. Jiang, Y., X. Duan, D. Joyce, Z. Zhang and J. Li, 2004. Advances in understanding of enzymatic browning in harvested litchi fruit. Food Chem., 88: 443-446.
33. Pariasca, J.A.T., T. Miyazaki, H. Hisaka, H. Nakagawa and T. Sato, 2001. Effect of Modified Atmosphere Packaging (MAP) and Controlled Atmosphere (CA) storage on the quality of snow pea pods (*Pisum sativum* L. Var. *saccharatum*). Postharvest Biol. Technol., 21: 213-223.
34. Martins, R.C., I.C. Lopes and C.L.M. Silva, 2005. Accelerated life testing of frozen green beans (*Phaseolus vulgaris*, L.) quality loss kinetics: Colour and starch. J. Food Eng., 67: 339-346.