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Studies on Chitosan and *Aloe vera* Gel Coatings on Biochemical Parameters and Microbial Population of Bell Pepper (*Capsicum annuum L.*) Under Ambient Condition

H.G. Manoj[1], K. N. Sreenivas[1]* T. H. Shankarappa[2] and H. C. Krishna[1]

[1]Department of Post Harvest Technology, College of Horticulture, GKVK, Bengaluru-560 065, India
[2]Department of Agricultural Microbiology, College of Horticulture, Tamaka, Kolar- 563 102, India

**Corresponding author*

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A B S T R A C T

The studies on chitosan and *Aloe vera* gel coatings on biochemical changes and shelf life bell pepper (*Capsicum annuum L.*) were carried out with different combinations. Chitosan 1 per cent showed better shelf life with bio chemical parameters such as, titratable acidity and total antioxidant activity. It had highest titratable acidity content 0.22 per cent, antioxidant activity of 204.94 mg100g^{-1}at 9 days of storage, it was much higher than other treatments. Chitosan @ 1 and 2 per cent also showed best result on microbial inhibition. The TSS and ascorbic acid contents were found to be decreasing over storage period. The gel extract from leaves of *Aloe vera* @ 20% indicated its inhibitory action on bacterial and mycelial growth of during ambient storage.

Introduction

Post-harvest losses of fruits and vegetables are a matter of grave concern in agriculture based country like India. Fruits and vegetables being highly perishable in nature demands at most care to minimize losses, the loss during post harvest operations range from 20-50 per cent in developing countries due to improper storage and handling (Kader, 1992).

Bell pepper is one of the most popular and high value commercial vegetable in India. It is a highly perishable with a short shelf life and susceptible to fungal diseases (Hardenburg *et al.*, 1990). Bell pepper had taken a pride of place among vegetables in Indian cuisine because of its delicacy and pleasant flavour coupled with rich content of ascorbic acid and other vitamins and minerals.

Recently, edible films have been developed to extend the shelf life of fruits and vegetables. The technology employs the film to be closely wrapped around the fruit controlling respiration and transpiration, thus slowing down senescence. The mechanism by which coatings preserve fruits and vegetables is by producing a modified atmosphere surrounding the product (Smith *et al*., 1987). The accumulation of CO_2 and depletion of O_2 is the principle behind MA packaging. MA packaging with chitosan and *Aloe vera* could also prolong shelf life of fruits and vegetables. The chitosan coating being non-toxic and biodegradable acts as a semi permeable barrier for exchange of gases and reduces water loss and microbial decay of harvested vegetables for extended periods (Dong *et al.,* 2004). *Aloe vera* gel based edible coating has been shown to prevent loss of moisture and firmness, control respiratory rate and maturation development. Therefore experiments were taken up to study the biochemical and microbiological changes that occur during the storage of bell pepper in response to coatings of chitosan and aloe vera gel.

Materials and Methods

The studies on chitosan and *Aloe vera* gel coatings on storage life of bell pepper (*Capsicum annuum L.*) were carried out at the Department of Postharvest Technology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Gandhi Krishi Vignana Kendra, Bengaluru campus, during 2013. Optimum matured disease free, green bell pepper fruits of Indra cultivar procured on the site of farm were subjected for investigation. The water soluble chitosan powder procured from the Pelican Biotech and Chemical Labs Pvt. Ltd, Kerala were used in the studies and the *Aloe vera* plants obtained from the Department of Medicinal and Aromatic crops of College of Horticulture, Bengaluru.

The water soluble chitosan powder was used at one and two per cent concentrations. 10 gm of chitosan was dissolved in 1000 ml of distilled water to obtain 1 per cent. 20 g chitosan was dissolved in 1000 ml of distilled water to obtain 2 per cent chitosan solution.

Fresh *Aloe vera* leaves were washed to remove dust, cut with knife and scooped to extract clean gel. The fresh gel was mixed thoroughly and strained through muslin cloth to remove thick particles. *Aloe vera* gel matrix was separated from the outer cortex of leaves and this colourless hydroparenchyma was grind in a blender. The liquid obtained constituted fresh *Aloe vera* gel. The gel matrix was pasteurized at 50^0C for 15 minutes.

The bell pepper fruits were dipped in soluble chitosan solution of 1 and 2 per cents and *Aloe vera* gel of 10 and 20 per cents for 10 minutes, air dried, kept under ambient condition in a room without package under aseptic condition. Nine treatments comprising i. control, ii.1% chitosan, iii. 2% chitosan, iv.10% *Aloe vera* gel, v. 20% *Aloe vera* gel, vi.1% chitosan + 10% *Aloe vera* gel, vii. 2% chitosan + 20% *Aloe vera* gel, viii. 1% chitosan + 20% *Aloe vera* gel and ix. 2% chitosan + 10% *Aloe vera* gel were prepared with five replications. Each replication had 500g of sample and observations for bio chemical parameters such as total soluble solids (TSS), total acidity, ascorbic acid, and anti oxidant parameters and microbial growth. The observations were recorded at 3, 6 and 9 days of storage and statistically analysed using complete randomized design.

The TSS of bell pepper fruit juice was

determined with the help of digital hand refractometer and expressed as degree Brix (^{0}B). The readings were corrected and adjusted to ambient temperature. Titrable acidity and ascorbic acid content of the fruit pulp was determined by titrating it with 0.1N sodium hydroxide and 2, 6-dichlorophenol indophenol titration method as suggested by Ranganna (2008) respectively. The total antioxidant capacity (mg/100g) was measured spectrophoto-metrically as suggested by Benzie and Strain (1996). The surface micro flora of bell pepper was studied for bacteria and fungi population on nutrient agar and potato dextrose agar for bacteria and fungi respectively using spread plate technique. The number of bacteria or fungi per gram of sample was expressed as colony forming units.

Results and Discussion

Total Soluble Solids (^{0}Brix)

The total soluble solids (TSS) content of bell pepper fruits did not differ significantly in any of the treatments until 3rd day (Table 1). It was found that the total soluble solids increased rapidly during the storage period. The highest being in untreated (control) fruits (4.87 ^{0}B, 5.13 ^{0}B, and 7.03 ^{0}B, as recorded on 3rd, 6th and 9th day of storage respectively). On the other hand, TSS content of fruits coated with 1 per cent chitosan was found to be low (4.17 ^{0}B, 4.17 ^{0}B, and 5.27 ^{0}B) amongst all the treated fruits. This treatment was followed by bell pepper fruits coated with 2 per cent chitosan (4.43 ^{0}B, 4.23 ^{0}B and 5.70 ^{0}B on 3rd, 6th and 9th day of storage respectively). Total soluble solids content of bell pepper fruits was found to increase considerably during storage in all treatments. Increase in TSS content of fruits might be attributed to increase in soluble solids, soluble pectin and soluble organic acids (Hamid Salari *et al*., 2012).

Titratable Acidity (%)

There was no significant differences between the treated and untreated bell pepper up to 3rd day, later days, the acidity in untreated fruits on 6th and 9th day of storage had decreased (0.19 % and 0.14 %) as compared to coated fruits, while the fruits coated with 1 per cent chitosan showed higher acid content (0.26 % and 0.22 %) on 6 and 9th days of storage respectively (Table 1). This treatment was followed by bell pepper fruits coated with 2 per cent chitosan at 0.25 % and 0.21 % on 6th and 9th day of storage respectively. This was mainly due to delayed ripening process caused by modified atmosphere of coated bell pepper fruits. It may be due to the ability of chitosan coating to modify internal atmosphere conditions, which caused reduced respiration and transpiration rate of coated fruits that could have slowly decreased the acid content (Gonzalez-Aguilar *et al*., 1999).

Ascorbic Acid (mg/100g)

Ascorbic acid content of the fruits had decreased during the storage period and was statistically significant (Table 2). Ascorbic acid content of untreated (control) fruits was found to decrease rapidly. It was lowest (107.87 mg100g^{-1}, 89.60 mg100g^{-1} and 74.73 mg100g^{-1}) as compared to coated fruits as recorded on 3rd, 6th and 9th day of storage. The fruits coated with 1 per cent chitosan had highest ascorbic acid content (113.60 mg 100g^{-1}, 99.20 mg100g^{-1}and 84.33 mg100g^{-1}) as recorded on 3rd, 6th and 9th days of storage. This was closely followed by fruits coated with 2 per cent chitosan (112.80 mg 100g^{-1}, 97.60 mg100g^{-1}and 80.67 mg100g^{-1}) on 3rd, 6th and 9th days of storage respectively.

The low decrease in ascorbic acid due to chitosan coating was mainly due to delayed ripening process because of modification of atmosphere or coated bell pepper fruits. It may also be due to the ability of chitosan coating to modify internal atmosphere conditions, which might have reduced respiration and transpiration rate of coated fruit, to impart slow decrease in the acid content. The purpose of the two selected coatings was to help preserve vitamin content and prevent oxidation. Exposure of vegetables to high temperatures and low humidity levels had deleterious effects on their nutritional quality. Vitamin C content had decreased with storage period (Hamid Salari *et al.,* 2012). In addition, pre treatments were found effective on preventing vitamin C degradation during storage period.

Total Antioxidant Activity (mg/100g)

Total antioxidant activity (fresh weight basis) of bell pepper fruits had increased during the storage period and was statistically significant among different treatments (Table 2). Total antioxidant activity of untreated (control) fruits was found to increase rapidly during storage. It was highest (144.94 $mg100g^{-1}$, 179.06 $mg100g^{-1}$ and 263.45 $mg100g^{-1}$) as compared to any coated fruits on 3rd, 6th and 9th days of storage. The fruits coated with 1 per cent chitosan had lowest antioxidant content (126.71 $mg100g^{-1}$,156.71 mg $100g^{-1}$ and 204.94 mg $100g^{-1}$) on 3rd, 6th and 9th days of storage. This was followed by 2 per cent chitosan (129.65 mg $100g^{-1}$, 158.47 mg $100g^{-1}$, and 217.88 $mg100g^{-1}$) on 3rd, 6th and 9th days of storage respectively.

Table.1 Effect of Chitosan and Aloe Vera Gel Coating on Total Soluble Solids (TSS) and Total Acidity of Bell Pepper Fruits Stored at Ambient Condition

Treatments	TSS (^{0} B)			Total Acidity (%)		
	3 days	6 days	9 days	3 days	6 days	9 days
T1: Control	4.87	5.13	7.03	0.24	0.19	0.14
T2: 1% Chitosan	4.17	4.17	5.27	0.28	0.26	0.22
T3: 2% Chitosan	4.43	4.23	5.70	0.26	0.25	0.21
T4: 10% *Aloe vera* gel	4.57	4.77	6.43	0.26	0.22	0.19
T5: 20% *Aloe vera* gel	4.47	4.50	5.87	0.27	0.24	0.20
T6: 1% Chitosan + 10% *Aloe vera* gel	4.50	4.60	6.07	0.26	0.23	0.18
T7: 2% Chitosan + 20% *Aloe vera* gel	4.70	4.80	6.10	0.26	0.22	0.16
T8: 1% Chitosan + 20% *Aloe vera* gel	4. 73	4.97	6.30	0.25	0.21	0.16
T9: 2% Chitosan + 10% *Aloe vera* gel	4.77	5.03	6.63	0.25	0.20	0.15
S. Em ±	0.08	0.09	0.20	0.01	0.01	0.01
CD @ 1%	NS	0.37	0.81	NS	0.04	0.04
	Initial TSS (^{0}B): 4.20			Initial acidity: 0.30		

Table.2 Effect of Chitosan and Aloe Vera Gel Coating on Ascorbic Acid Total Antioxidant Activity (TAA) of Bell Pepper Fruits Stored at Ambient Condition

Treatments	Ascorbic acid (mg 100 g^{-1})			TAA (mg 100 g^{-1})		
	3 days	6 days	9 days	3 days	6 days	9 days
T1: Control	107.87	89.60	74.73	144.94	179.06	263.45
T2: 1% Chitosan	113.60	99.20	84.33	126.71	156.71	204.94
T3: 2% Chitosan	112.80	97.60	80.67	129.65	158.47	217.88
T4: 10% *Aloe vera* gel	110.40	94.40	78.00	135.53	164.94	243.18
T5: 20% *Aloe vera* gel	112.40	96.00	79.47	130.82	162.00	234.35
T6: 1% Chitosan + 10% *Aloe vera* gel	110.13	92.07	78.47	133.18	163.76	238.00
T7: 2% Chitosan + 20% *Aloe vera* gel	109.60	91.93	78.40	137.88	167.29	247.45
T8: 1% Chitosan + 20% *Aloe vera* gel	109.33	91.20	77.60	140.24	172.59	254.94
T9: 2% Chitosan + 10% *Aloe vera* gel	108.67	90.40	76.67	141.16	175.75	257.29
S. Em ±	0.89	0.71	1.19	0.11	0.12	0.13
CD @ 1%	3.60	2.87	4.84	0.45	0.51	0.53
	Initial ascorbic acid: 114.20 mg 100 g^{-1}			Initial TAA: 124.35 mg100 g^{-1}		

Table.3 Chitosan and Aloe Vera Gel Coating on Microbial Population of Bell Pepper Fruits Stored at Ambient Condition at 9 Days of Storage

Treatments	CFU g^{-1}	CFU g^{-1}
	Bacteria	Fungi
T1: Control	1.19 X 10^3	1.2 X10^3
T2: 1% Chitosan	0.28 X 10^3	0.39 X 10^3
T3: 2% Chitosan	0.47 X 10^3	0.53 X 10^3
T4: 10% *Aloe vera* gel	0.11 X 10^3	0.18 X 10^3
T5: 20% *Aloe vera* gel	0.09 X 10^3	0.15 X 10^3
T6: 1% Chitosan + 10% *Aloe vera* gel	0.29 X 10^3	0.66 X 10^3
T7: 2% Chitosan + 20% *Aloe vera* gel	0.58 X 10^3	0.7 X 10^3
T8: 1% Chitosan + 20% *Aloe vera* gel	0.6 X 10^3	0.8 X 10^3
T9: 2% Chitosan + 10% *Aloe vera* gel	0.83 X 10^3	0.9 X 10^3
S. Em ±	2.22	3.33
CD @ 1%	9.05	13.57

Antioxidant activity of bell peppers depends on several factors including genetic, environmental condition (temperature, light, water, and nutrient availability), production techniques used (plant grow regulators, date of harvest, etc.) and post harvest storage conditions (Sun *et al*., 2007). Increasing TAA during storage could be related to

ripening processes and metabolism of phenolic compounds. The increase in TAA is mainly due to changes in to the LAA. Carotenoid content in pepper fruits also increases during storage condition. Temperature during storage conditions affect the pathways involved in the biosynthesis secondary metabolites, lead to higher phenolic metabolism and antioxidant capacity on pepper fruit.

Microbial Population

The microbial population in bell pepper fruits as influenced by different concentrations of chitosan and *Aloe vera* gel showed variation in population during the storage period. The uncoated (control) fruits had maximum bacteria population of 1.19 X 10^3 $CFUg^{-1}$ at the end of the storage (9^{th} day), whereas the fruits coated with 20 per cent *Aloe vera* gel showed lowest population 0.09 X 10^3 $CFUg^{-1}$. This treatment was followed by fruits coated with 10 per cent *Aloe vera* gel 0.11 X 10^3 $CFUg^{-1}$ on 9^{th} day of storage. The fungal population in bell pepper fruits as influenced by different concentrations of chitosan and *Aloe vera* gel showed differences in population during the storage period. The uncoated (control) fruits had maximum 1.2 X 10^3 $CFUg^{-1}$ at the end of the storage (9^{th} day) whereas the fruits coated with 20 percent *Aloe vera* gel showed minimum 0.15 X10^3 $CFUg^{-1}$. This treatment was followed by fruits coated with 10 per cent *Aloe vera* gel 0.18 X 10^3 $CFUg^{-1}$ on 9 day of storage.

The microbiological profile of the surface-coated bell pepper showed significant variation between the coated and uncoated samples. *Aloe vera* gel appears to contain various antibiotic and antifungal compounds that can potentially delay or inhibit microorganisms that are responsible for food borne illness in humans as well as food spoilage (Liu *et al*., 2004). Antimicrobials of a plant source, such as the *Aloe vera* plant, might prove safer alternatives replacing synthetic chemicals. Chitosan 1 and 2 per cent also showed best results with respect to microbial inhibition when compared control.

In conclusion, the TSS content of chitosan 1 per cent coated bell pepper fruits had lowest TSS content and showed highest acid content at the end of 9 days storage. Chitosan 1 per cent coated bell pepper fruits had lost minimum amount of ascorbic acid. The total antioxidant content of bell pepper fruits increased with increasing ripening process, untreated bell pepper fruits had maximum amount of antioxidant activity at 6 and 9 days of storage, the fruits coated with 1 per cent chitosan showed lower amounts of antioxidant activity. The results also indicate that the gel extract from leaves of *Aloe vera* inhibited the bacterial and fungal growth of common postharvest mcroorganisms.

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