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Effect of chitosan coating on storage stability of tomatoes (*Lycopersicon esculentum Mill*)

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Abstract

Tomato (*Lycopersicon esculentum*) is one of the most widely consumed fresh fruity vegetables in the world. Tomatoes were coated with 0.25 and 0.5% chitosan. The physico-chemical parameters and microbial load were studied for a period of 30 days. The lower concentrations of chitosan 0.25% was found effective than 0.5% in maintaining the physico-chemical characteristics. The results clearly indicated that fruits treated with chitosan were better in maintaining all physicochemical characteristics (pH-4.7, TSS-16.03, Acidity-0.35, Ascorbic acid-23.54, Weight loss-7.34 and Moisture-93.96) and better reduction in microbial growth (Total plate count-18.16, yeast and mold-5.12 cfu/log) sensory attributes (Appearance-4.43, Taste-4.06 and Flavour-4.43) than control throughout the storage period. The study concludes that chitosan coating could be a good alternative to preserve the quality and extend the post-harvest life of tomatoes.

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Introduction

Tomatoes were ranked highest in a comparison of vegetable crops and their contribution of nutrients especially good sources of vitamins and minerals to the diet which provides health benefits (Peralta and Spooner, 2006) and is well popular vegetable fruit used in all type of culinary systems throughout the world. Fruits have wide range of nutritional properties and are easily mingled with all type of food preparations. Tomato products such as sauce, ketchup and puree are chiefly depended on the production and quality of tomatoes. Ripen tomato fruits are very perishable and liable to transport damage that consequently leads to loss of quality and quantity. This is especially quite common in developing countries due to poor post harvest handling systems, storage facilities and transportation. Losses during post harvest operations due to improper storage and handling are enormous and can range from 20-50 percent in developing Countries (Kader and Rolle, 2004). By utilizing the improved post harvest practices often results in reduced losses, improved overall quality and food safety and higher profits for growers and marketers (Kitinoja and Kader, 2002). Edible coating (EC) is defined as a thin layer of material formed on the surface of the food for the purpose of preservation and can be eaten whole with the food. EC act as a barrier against transmission of gases, vapors, solutes and also provides mechanical protection to the foods (Gontard and Guilbert, 1994; Wu *et al*., 2002). The application of edible coatings becomes the most advanced method to extend the shelf life of the fresh produce by regulating its metabolic activities. Edible coatings with their unique barrier, anti-microbial nature extend the shelf life; enhance the quality and microbial safety of fresh and minimally processed fruits and vegetables (Lin and Zhao, 2007).

EC components can be divided into three categories: hydrocolloids, lipids and composites. Hydrocolloids include proteins and polysaccharides such as starch, alginate, cellulose derivatives, chitosan and agar. Lipids include waxes, acylglycerols and fatty acids. Composites contain both hydrocolloid and lipid components (Espino-Diaz *et al*., 2010). The material for film or coating is largely dependent on its desired function. Chitosan (poly-β-(1→4) N-acetyl-D-glucosamine) is a bio-polysaccharide isolated from the outer shell of crustaceans has become an effective alternate for biocidal treatment due to its natural character, antimicrobial activity (Terry and Joyce, 2004). Chitosan is a biodegradable cationic polymer with potential antimicrobial and film forming agent (Jung *et al*., 1999; No *et al*., 2002; Tharanathan and Kittur, 2003; Zheng and Zhu, 2003; Kong *et al*., 2010).

The toxicological study by Hirano *et al*. (1990) indicated Chitosan as a byproduct from the seafood industry appears to be a safe material. When fruit is coated with chitosan, it forms a semi permeable film

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and modifies the internal atmosphere of the tissue and consequently delays ripening (Bai *et al*., 1988). They also used to inhibit migration of moisture, O_2 and CO_2 in foods and compatibility with the other substances like vitamins, minerals and antimicrobial agents (Krochta and De Mulder-Johnston, 1997). The use of chitosan based polysaccharide films in food wrapping has been expanded due to its unique physical, chemical and film forming properties (Krochta, 1997; Shahidi *et al*., 1999; Han *et al*., 2004; Park and Zhao 2004; Han *et al*., 2005; Durango *et al*., 2006; Vargas *et al*., 2006; Chien *et al*., 2007; Ribeiro *et al*., 2007).

Edible coatings are available mainly to preserve the quality of fruits especially citrus and apples and to minimum extent to mangoes, papayas, pomegranates, cherries, avocados, cantaloupes and tomatoes among the fruits (Olivas *et al*., 2008). However the preservation of the whole tomato fruits with edible coatings has been used related to the studies and reviews found on the topic of edible coatings for whole fruits and vegetables (Claypool, 1940; Park and Chinnan, 1990; Hagenmaier and Shaw, 1992; Banks *et al*., 1993; Baldwin, 1995; Park, 1999; Maftoonazad *et al*., 2008; Perez-Gago *et al*., 2010). Due to the high perishability, to extend the storage life and its importance in world agricultural trade, present study was planned to evaluate the effect of chitosan edible coating on shelf life of tomatoes. Physicochemical parameters and microbial growth were analyzed periodically to understand the qualitative and quantitative changes in tomatoes during the storage.

Materials and Methods

Materials

Fresh mature and reddish green tomatoes (*Lycopersicon esculentum* mill) were collected from the local farmers with uniform size, shape, color, maturity and without any signs of mechanical damage or fungal decay. Chitosan was purchased from Sisco Research Laboratories and glacial acetic acid from Merck India Ltd.

Preparation of coat forming solution

The coating solution was prepared by dissolving 2.5 and 5 g of chitosan powder in 900 ml of distilled water, 50 ml of glacial acetic acid was added to dissolve the chitosan to prepare 1 L of 0.25%, 0.5% chitosan solutions (Jiang and Li, 2001) and pH was adjusted to 5.0 with 0.1M NaOH and the solution was made up to 1L. The coating solutions was prepared and coded as control, 0.25% and 0.5% chitosan solutions. The acid solution of pH 5.0 without chitosan was prepared and used as control.

Application of coating

The surface of the fruits were disinfected with 4% chlorine (hypochlorite) for 3 min and gently rinsed with distilled water, then air-dried. Fruits were separated into three groups in triplicate; each group of the fruits was quoted as Control (without treatment), 0.25% and 0.5% chitosan coating. Each group of tomatoes was divided into 12 batches in triplicate (36 batches) each containing 100-110g of whole tomatoes. They were dipped in the chitosan coat forming solution of 0.25% and 0.5% for 1 min and the samples were air dried for 30 min at room temperature (Approx 30°C).The coated fruits were packed in cost effective locally available thermo bowls and tightly over wrapped by using PVC wrap film and kept at 6°C in a refrigerated condition for a period of 30 days to study the shelf life and physico-chemical and microbial parameters.

Determining weight loss and moisture content

Three batches of tomatoes containing 100-110g of whole tomatoes were taken at an interval of three days for total storage period. The tomatoes were weighed regularly to determine weight loss, which was calculated cumulatively by comparing the weights of the sample with the electronic weighing balance (Shimadzu- ELB300 NO: D515711067, Japan) at an interval of 2 days for the total 30 days storage period and the results were expressed as percentages. The moisture content was determined by the method (Williams, 1984).

Measurement of pH, total soluble solids, titratable acidity and ascorbic acid

The pH, total soluble solids (TSS) and titratable acidity (TA) have been determined by the methods followed by Islas-osuna *et al*. (2010) with slight modifications. 5 g tomato pulp was homogenized in 25 ml of distilled water. Then the mixture was filtered using muslin cloth. An aliquot of 25 ml was used to measure pH with a pH meter (Eutech instruments, prod- ECPH70042SEU, Singapore).

The TSS was measured directly from the filtered residue using a hand refractometer (Erma Inc. Tokyo, Japan) and expressed as brix^0.The titratable acidity was determined with 0.1 N NaOH. Tomato pulp (3g) from fruit was homogenized using a mortar and pestle (grinder) and then centrifuged at 3500 rpm(Remi centrifuge, CE model, India) for 10 minutes; The supernatant phase was collected and analyzed to determine ascorbic acid content by

2,6-dichlorophenolindophenol titration (Williams, 1984).

Microbial growth

The total colony forming units (CFU) was enumerated during the storage period. 10 g of sample was obtained after homogenization in 90 ml ringer's solution; other decimal dilutions were prepared from a 10^{-1} dilution. The total count was assessed using the pour plate method and prepared plate count agar as culture media (HiMedia, M001). Plates were incubated at 35°C for 48 hrs. Samples were analyzed from 0 to 30th day at an interval of 2 days and microbial counts were expressed as log CFU/g (Mccance and Harrigan, 1976).Similarly the yeast and mold count was assessed using the pour plate method by dextrose agar as the culture media (HiMedia,M403). Plates were incubated at 30°C±2°C for 3 to 5 days (72 hrs) and expressed as CFU/g of the sample.

Sensory evaluation

The acceptability of the samples was evaluated through the standard sensory evaluation techniques. The sensory attributes such as visual appearance, color, taste, flavor and acceptability was carried out by selected panel of judges (10 Members) rated on a five point hedonic scale (5-Excellent, 4-Very good, 3-Good, 2-Fair, 1-Poor).

Statistical analysis

Statistical analysis was carried out in three replicates for the control and experimental samples. The data has been analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test, Duncan's multiple range test for the average value of parameter among the three treatments and used to compare the mean values between pair of treatments. Differences were calculated to compare significant effects at $p \leq 0.05$ level.

Results and Discussion

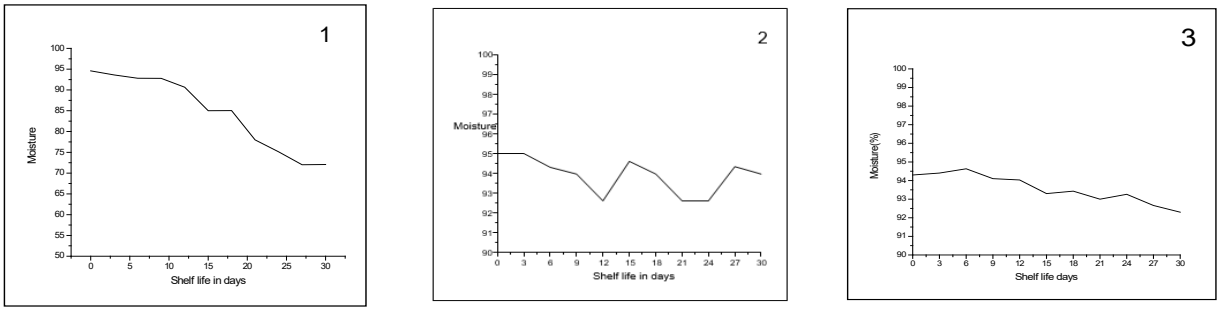
Weight loss and Moisture content

Chitosan coatings controlled the weight loss of tomatoes compared to control (Table 1). After 30 days of storage, the weight loss of the control, 0.25% and 0.5% coated tomatoes were 14.88%(highest), 7.34%(lowest) and 9.28%(lowest) respectively. The weight loss observed in control was due to the shrinkage of fruits by loss of moisture which was not observed in the coated fruits. The chitosan coating prevented the evaporation of moisture from coated tomatoes. There was a significant difference observed between the control and coated samples. The 0.25% coated tomatoes showed better retention in moisture when compared with control and 0.5% coated tomatoes at the end of the storage period (Figure 1).

Table 1. Effect of chitosan coating on weight loss of whole tomato stored at 6°C

Days (Storage period)	Chitosan concentration (%)		
	Control	0.25	0.5
	Weight loss %		
0	0	0	0
3	2.1±0.80^{a}	0.22±0.46^{b}	0.34±0.43^{b}
6	3.9±0.46^{a}	0.54±0.46^{b}	1.30±0.46^{b}
9	5.1±0.46^{a}	1.42±0.46^{b}	2.30±0.46^{b}
12	6.24±0.46^{a}	2.24±0.46^{b}	3.52±0.46^{b}
15	7.92±0.46^{a}	3.24±0.46^{b}	3.98±0.46^{b}
18	8.46±0.46^{a}	4.1±0.46^{b}	4.60±0.46^{b}
21	10.0±0.46^{a}	5.2±0.46^{b}	5.74±0.46^{b}
24	12.30±0.46^{a}	5.98±0.46^{b}	6.90±0.46^{b}
27	13.54±0.46^{a}	6.36±0.46^{b}	7.98±0.46^{b}
30	14.88±0.46^{a}	7.34±0.46^{b}	9.28± 0.46^{c}

Means followed by the same letter in the row are not significantly differ by Tukey's test ($p>0.05$). Each trial contained three replicates of (100-110g) whole tomatoes each per treatment. Weight loss was evaluated at a 2 days interval for 30 days of storage period. (Control, 0.25% Chitosan and 0.5%Chitosan)



Means followed by the same letter in the row are not significantly differ by Tukey's test ($p>0.05$). Each trial contained three replicates of (100-110g) whole tomatoes each per treatment. Moisture was evaluated at a 2 days interval for 30 days of storage period (Control, 0.25% Chitosan and 0.5%Chitosan).

Figure 1. Effect of chitosan coating on moisture content of whole tomato stored at 6°C Moisture loss: (1) Control sample (2) 0.25 % chitosan, (3) 0.5% chitosan.

Table 2. Chitosan coating analyses on whole tomato after thirty days of storage period at 6°C

	Parameters			
Chitosan (%)	Total soluble Solids (°Brix)	Titratable acidity (%)	pH	Ascorbic acid
Before treatment	17.63±0.47	0.85± 0.46	4.13±0.18	33.2±0.50
After storage				
15th Day				
Control	17.80±0.30[a]	0.35±0.44[a]	5.08±0.46[a]	16.58±0.49[a]
0.25	14.00±0.34[b]	0.38±0.44[a]	4.55±0.46[a]	26.66±0.46[b]
0.5	18.00±0.46[a]	0.70±0.46[a]	4.65±0.46[a]	16.61±0.45[a]
30th Day				
Control	21.03±0.08[a]	0.34±0.25[a]	5.25±0.13[a]	16.44± 0.50[a]
0.25	16.03±0.06[b]	0.35±0.29[a]	4.70±0.46[a]	23.54±0.61[b]
0.5	18.02±0.06[c]	0.37±0.34[a]	4.86±0.25[a]	16.44± 0.46[a]

Means followed by the same letter in the row are not significantly differ by Tukey's test (p>0.05). Each trial contained three replicates of (100- 110g) whole tomatoes each per treatment was evaluated at an interval of 2 days for 30 days of storage period (Control, 0.25% Chitosan and 0.5%Chitosan).

Total soluble solids, titratable acidity, pH and ascorbic acid

The total soluble solids (TSS) content showed a significant difference between the control and 0.25% sample but not significant between control and 0.5% coated tomatoes during 15th day of storage. Consequently at the end of storage period there was a significant difference observed between the coated and uncoated samples. It is expected to increase during ripening and decrease during storage (Tasdelen and Bayindirli, 1988).The titratable acidity of tomato fruit fell after thirty days of storage (Table 2).But the titratable acidity contents and pH did not vary significantly among the fruits treated with control, 0.25% and 0.5%.The titratable acidity of the tomatoes decreased with maturity and was not significantly affected (p>0.05) by coating treatment.

The same results were observed in a study by Raffo *et al*. (2002) which shows the acidity decreased with maturation and increased with high percent of sugar content in fruit. The increase in pH shows that organic acids provide most of the hydrogen ions in tomatoes and normally decrease with ripening produce an increase in pH. The physico-chemical parameters like total soluble solids, titratable acidity, pH may also influenced by factors such as cultivar, cultural practices, region of cultivation and season (Suarez *et al*., 2008) .The ascorbic acid content of the whole tomato fruit decreased after 30 days of storage (Table 2).The tomato fruit that has been treated with chitosan (0.25%) has a greater retention of ascorbic acid content. There was a significant difference in vitamin-C content between the 0.25% coated with control and 0.5% coated samples.

Sensory evaluation

Chitosan coating improved the sensory quality and extends the shelf life of tomatoes when compared to control. Both the control and the chitosan coated (0.25 and 0.5%) whole tomatoes were still commercially satisfactory in color, taste, flavor, appearance and overall acceptability after they had been stored for 15 days. The control sample spoiled and completely unacceptable on 20th day, whereas chitosan coated samples retained their quality up to the end of storage period (30th day) without any spoilage and acceptable in all sensory parameters. The sensory quality up to 15 days did not vary among the fruit treated with chitosan and control samples. The control sample started deteriorated from 20th day and spoiled on 24th day of the storage period. At the end of storage period, there was no significant difference in all sensory aspects observed between the coated samples 0.25 and 0.5% (Table 3).

Table 3. Effect of Chitosan coating on sensory quality of whole tomato after thirty days of storage period at 6°C

Sensory attributes	Chitosan concentration (%)		
	Control	0.25	0.5
Color			
0 Day	5	5	5
15 Days	3.86± 0.35[a]	3.70± 0.26[a]	3.86± 0.35[a]
30 Days	*	3.86 ±0.29[c]	3.93± 0.40[c]
Appearance			
0 Day	5	5	5
15 Days	3.76 ±0.18[a]	4.40±0.46[a]	3.86±0.35[a]
30 Days	*	4.43±0.34[b]	3.50±0.28[b]
Taste			
0 Day	5	5	5
15 Days	3.75± 0.29[a]	4.20±0.41[a]	3.73±0.17[a]
30 Days	*	4.06±0.32[b]	3.46±0.37[b]

Table 3 (Cont.)

Flavor			
0 Day	5	5	5
15 Days	3.70±0.25[a]	3.80±0.15[a]	3.70±0.17[a]
30 Days	*	4.43± 0.44[b]	3.70±0.17[b]
Overall acceptability			
0 Day	5	5	5
15 Days	3.00±0.30[a]	3.31±0.30[a]	3.26±0.34[a]
30 Days	*	3.70±0.26[b]	3.53±0.34[b]

Means followed by the same letter in the row are not significantly differ by Tukey's test ($p>0.05$). Each trial contained three replicates of (100-110g) whole tomatoes each per treatment. Sensory quality was evaluated at an interval of 2 days for 30 days of storage period. (Control, 0.25% Chitosan and 0.5%Chitosan)

*sample attained maximum deterioration

Table 4. Effect of chitosan coating on microbiological changes of whole tomato stored at 6°C.

Chitosan concentration (%)	Total Plate Count	Yeast and mould count
Before treatment		
Control	2.27[a]	1.67[a]
0.25	2.27[a]	1.67[a]
0.5	2.27[a]	1.67[a]
After treatment		
15th day		
Control	8.39[a]	5.17[a]
0.25	7.99[b]	5.07[a]
0.5	8.15[c]	5.30[a]
30th day		
Control	*	*
0.25	8.16[a]	5.12[a]
0.5	8.23[a]	5.26[b]

Means are averaged values of three trials (n = 3; mean value ± standard error). Each trial involved three identical groups of 100-110g whole tomato per treatment. Microbiological analysis was conducted at an interval of 2 days for 30 days of storage period. Values within a column with the same letter are not significantly different ($p > 0.05$(Control, 0.25% Chitosan and 0.5%Chitosan)

* Sample attained maximum deterioration.

Microbial analysis

The results of microbiological analysis of coated and uncoated tomato samples were given in (Table 4).The total bacterial count of control sample increased from 2.27 to 8.39 log cfu/g on 15th day of storage period and on 24th day it was completely deteriorated. On day 15th there was a significant difference in microbial count observed between the chitosan coated samples. At the end of the storage period there was no significant difference observed in microbial load between the coated samples.

The yeast and mold count of the samples shows that there was no significant difference ($p>0.05$) recognized between the coated and uncoated samples. The total yeast and mold count of control sample increased from 1.67 to 5.17 log cfu/g on 15th day of storage period and on 24th day it was also completely deteriorated. At the end of the storage period there was a significant difference observed between the chitosan coated samples (0.25 and 0.5%). The chitosan coating on whole tomato effectively inhibited the growth of microorganisms.

Conclusion

The physical, chemical and microbial results revealed that chitosan coated tomatoes are good in extending shelf stability up to 30 days at 6°C storage. Chitosan coating of tomatoes can be employed to improve the shelf stability by inhibiting the microbial spoilage and also retains its maximum sensory attributes during storage at lower temperatures. In conclusion, to increase the shelf life of the tomatoes, chitosan coatings can be considered for commercial application to extend the storage period of fresh produce.

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