Application of chitosan edible coating for preservation of tomato

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Abstract
Tomato is one of the most widely consumed fresh fruit in the world. It is extremely perishable. Edible coatings serve as the best possible alternative to reduce the post-harvest losses by delaying the ripening of tomato and increasing the shelf life without affecting the quality. Tomatoes were coated by dipping method with different concentrations of chitosan. The effect of this coating on TSS, pH, titratable acidity, shrinkage and lycopene content of tomato were investigated for a period of 30 days at ambient temperatures. Coated tomatoes were firmer, higher in titratable acidity, less decayed. Change in pH and TSS were observed to be less in coated samples than the control fruit at the end of storage. On 20th day of storage, lowest TSS value (5.1% Brix) was recorded in 2.5% chitosan and the highest TSS value (6.8% Brix) was recorded in control. The tomato in control showed a rapid deterioration from 20th day of storage. On the contrary, the coating on tomatoes delayed the ripening and extended the shelf life up to 30 days. From the results, it was concluded that 2.5% chitosan treatment resulted in better extension of the shelf life of tomato on storage when compared 0.5%, 1%, 2% chitosan coatings.

Keywords: Tomato, edible coatings, chitosan, post-harvest losses

Introduction
Huge post-harvest losses of fruits and vegetables are a matter of concern for many countries whose economy is agriculture based. Fruits and vegetables are highly perishable commodities that require to be handled with much care to minimize losses. Because of the high moisture content, agricultural crops are inherently more liable to deteriorate especially under tropical conditions. They are biologically active and carry out transpiration, respiration, ripening and other biochemical activities, which result in quality deterioration. To tackle these, edible coatings are the best possible solution.

Tomato is a climacteric fruit and continues to ripen after harvest. During ripening, the green pigment chlorophyll degrades and carotenoids are synthesized. For fresh tomatoes, the two quality attributes that are most important to buyers and consumers are texture and skin color. Texture is influenced by flesh firmness and skin strength. Softening during storage, distribution and ripening of tomatoes can be a major problem because it may increase their susceptibility to damage. Hence there is increasing consumer concern to preserve the eating quality of tomatoes (Batu, 2004) [3].

Chitosan is derived from chitin; it is an edible polymer, isolated from crustacean animal shells. It is a natural product which is non-toxic and eco-friendly. Chitosan, a high molecular-weight cationic polysaccharide produced by deacetylation of chitin, is applied widely in postharvest treatments because of its excellent film forming, antifungal, antibacterial and biochemical properties. Recently chitosan has attracted notable interest due to its biological activities, including antimicrobial (Tsai et al., 2004) [15], antitumor (Tokoro et al., 1988) [13], antioxidative (Lopez-Caballero et al., 2005) [7], and hypocholesterolemic functions (Sugano et al., 1992) [12] and it has bacteriostatic and bactericidal properties. For this reason, chitosan is a highly recommended polymer for the production of edible film coatings (Chien et al., 2007) [4].

In view of these, the project was undertaken to extend the shelf life of the tomatoes and decrease the post-harvest losses by the application of chitosan, with the objectives to extract the Chitosan from shrimp shells, study the effect of coatings applied on tomatoes by dipping method and to conduct shelf life studies.
Material and Methods

Experimental materials
The raw materials used are tomatoes of (9005 Siri) variety were procured from local farm, Kotagiri, Nizamabad dist. Shrimps were procured from local market, Bodhan, Nizamabad dist, Telangana state. The chemicals such as Hexane, Acetone, Ethanol, HCl, NaOH, Glycerol, Ascorbic acid, Citric acid and Phenolphthalein indicator for shelf life study were purchased from M/s Telangana scientific Pvt Ltd, Hyderabad, Telangana state.

Preparation of Chitosan
Fresh shrimp was collected from local market. Shrimp head and skin was separated from shrimp using sharp knife. The collected shrimp wastes were then washed with tap water and crushed with mortar pestle. Crushed shrimp waste was kept in a polyethylene bags at ambient temperature (28±2 °C) for 24 h for partial autolysis to facilitate chemical extraction of chitosan and to improve the quality of chitosan. Then isolation of chitosan was carried out using the following 3 (three) steps, namely demineralization, deproteinization and deacetylation. Demineralization of shrimp shell has been carried out with 3% HCl at ambient temperature (28±2 °C) with a solid to solvent ratio 1:5 (w/v) for 16 h. The residue was washed and soaked in tap water until neutral pH was obtained. Deproteinization of shrimp shell was done with 4% NaOH at ambient temperature (28±2 °C) with a solid to solvent ratio 1:5 (w/v) for 20 h. The residue was washed and soaked in tap water until neutral pH was obtained. Then purified chitin was dried. Chitin flakes were ground into smaller size particles to facilitate deacetylation for removal of acetyl groups from chitin. Deacetylation was experimented using four different concentration of NaOH (30%, 40%, 50%, 60%) at 65 °C temperature with a solid to solvent ratio 1:10 (w/v) for 20 h (Toan, 2009) 

Preparation of Chitosan solution
Shrimp shell chitosan solutions with concentrations C₁-0.5%, C₂-1%, C₃-2% and C₄-2.5% were prepared by adding 0.6% acetic acid and 25% glycerol (w/w chitosan). Each of the solutions were thoroughly mixed, filtered and the pH was adjusted to 5.6 using 1M sodium hydroxide (Park et al., 2004) [9].

Application of coating solutions
Mature Green Tomatoes variety (9005 Siri) were procured from local farm, Kotagiri, Nizamabad. Tomatoes were properly sorted to discard the tomatoes mechanically damaged while transportation. The procured tomatoes were washed thoroughly with running water and surface dried before coating for proper adherence of coating solutions on the surface of the tomato (Athmaselvi et al., 2013) [12]. The fresh fruits were dipped in the coating solutions at room temperature for 1 min. At regular intervals, the fruits were rotated for uniform application of coating. They were then allowed to dry at room temperature. Weights of the coated fruits were taken. The fruits were stored at room temperature (30 ± 3 °C). The experiment was done in triplicates.

Physico-chemical analysis
The following physico-chemical analysis was carried out for the tomatoes to assess the effect of coating solutions on the quality attributes of tomato.

Shrinkage percentage
The weight measurement on shrinkage of tomato fruit on n-th day of storage was done using the following equation.

\[
\text{Weight of shrinkage} = \frac{\text{fruit weight on 0th day} - \text{fruit weight on nth day}}{\text{fruit weight on 0th day}}
\]

Total soluble solids (% Brix)
Total soluble solids (TSS) were measured by the procedure given by Dong et al., (2001) [6]. Individual tomato fruit from each treatment will be ground in an electric juice extractor for freshly prepared juice. Soluble solids content was measured using Digital hand held pocket Refractometer (ATAGE) in % Brix. The range of the refractometer is 0 to 85%.

pH
Tomatoes were cut into small pieces and ground. 10 g of ground tomato sample was suspended in 100 ml of distilled water and then filtered. The filtered sample was used for assessment of pH using a pH meter (ATAGE).

Titratable acidity
Titratable acidity was determined according to the procedure of AOAC (2000) [1]. Five grams of tomato juice diluted in 25 mL of distilled water, two drops of phenolphthalein indicator and titrated by 0.1N sodium hydroxide (NaOH). The titratable acidity was expressed as g citric acid/kg tomato, according to the following equation:

\[
\text{Titratable acidity (g citric acid/kg tomato)} = \frac{(V \times 0.1 \times 1000 \times 0.064)}{m}
\]

Where, 0.1 is the normality of NaOH (N)
0.064 is the conversion factor for citric acid
V is the volume of NaOH required (mL)
m is the mass of tomato juice sample used (g)

Lycopene content
Fresh tomato juice was carefully weighed (4±0.01 g) into a 200 mL flask wrapped with aluminium foil to protect it from exposure to light. A 100 mL mixture of hexane-acetone-
ethanol, 2:1:1 (v/v %), was added to the flask and agitated continuously for 10 min on shaking water bath. Thereafter 15 mL of water was added followed by agitation for another 5 min. The solution was then left for separation into distinct polar and non-polar layers and filtered using filter paper (Whatman grade 42). Lycopene concentration was estimated by measuring the absorbance of the extract at 503 nm by UV-VIS Spectrophotometer using hexane as a blank (Ranveer et al. 2013) [10]. The lycopene concentration was calculated using its specific extinction coefficient (E1%, 1 cm) of 3120 in hexane at 503 nm. The lycopene concentration was expressed as mg/kg fresh tomato, and calculated by the following formula:

\[
\text{Lycopene (mg/kg fresh wt.)} = \frac{(A_{503} \times 537 \times 100 \times 0.55)}{(4 \times 172)} = A_{503} \times 42.9
\]

Where,
- 537 g/mole is the molecular weight of lycopene
- 100 mL is the volume of mixed solvent
- 0.55 is the volume ratio of the upper layer to the mixed solvents
- 4 g is the weight of tomato added
- 172 mM-1 is the extinction coefficient for lycopene in hexane

**Results and Discussion**

**Changes in Total Soluble Solids (TSS)**

The effect of chitosan concentration on TSS is shown in the Fig. 1. TSS increased upon storage. The highest Brix was observed on 30th day of storage period compared with the 0th day. The lowest TSS was observed at the 0th day. On 20th day of storage, lowest TSS value (5.1% Brix) was recorded in 2.5% chitosan and the highest TSS value (6.8% Brix) was recorded in control. The control fruit started deteriorating after 20th day of storage whereas the shelf life of majority of the coated fruits was extended to 30 days. Therefore, data for the uncoated fruit was given only till 20th day of storage.

**Changes in pH**

The effect of chitosan concentration on pH is shown in the Fig. 2. The highest pH was observed on 30th day of storage period compared with the 0th day. On 20th day of storage the lowest pH value (4.03) and the highest pH value (4.43) were recorded in 2.5% chitosan and control respectively. The increase in pH is due to the organic acids which provide most of the hydrogen ions in tomatoes and normally decrease with ripening produce an increase in pH.

**Changes in Shrinkage (%)**

The effect of chitosan on tomato during storage is shown in the Fig. 3. Chitosan coatings controlled the weight loss of tomatoes compared to control. Shrinkage in the uncoated tomato increased from 0 to 30.57% upon 20 days of storage. On 30th day of storage the shrinkage of 2.5%, 2%, 1%, 0.5% chitosan coated samples were 13.41%, 17.38%, 20.26%, 25.98% respectively. Hence chitosan 2.5% concentration showed less shrinkage when compared to other treatments. The excess 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.

**Changes in Titratable acidity (%)**

The titratable acidity (TA) of the tomatoes decreased with maturity. The same results were observed in a study by Raffo et al., (2002) [11] which shows the acidity decreased with maturation. In general, the decrease in acidity over time seems more pronounced in uncoated tomatoes compared with coated tomatoes and may be related to high ethylene production and respiration rate during ripening (Das et al., 2013; Oz and Ulukanli, 2011) [5, 8]. Effect of chitosan on TA% of tomatoes on storage for 30 days at every 5 days interval was shown in the Fig. 4.
Changes in Lycopene content (mg/kg)
The changes in lycopene content of chitosan coated and uncoated tomatoes is shown in the Fig.5. During ripening the chlorophyll content decreased, and there was a rapid synthesis of the red pigment lycopene. The lycopene content of control during its red stage on the 20th day was 44.09 mg/kg, whereas lycopene of 2.5% chitosan coated fruit on the same day was 4.18 mg/kg. The lycopene content of tomato increased during its ripening. The lycopene content of 2.5% chitosan coated fruit during its red stage on the 30th day was 33.23 mg/kg which is lower than the 0.5%, 1%, 2% treatments. It indicates that 2.5% concentration chitosan treatment was much effective in delaying the ripening and extending the shelf life of tomatoes.

Conclusion
The TSS content in all treatments generally increased with storage in the study. The TSS of the control fruit during the red stage on 20th day was 6.8% Brix whereas for the 2.5% chitosan it was 5.1% Brix. On 30th day the control fruit is deteriorated whereas the TSS for the 2.5% chitosan was 5.7% Brix. The pH value of all the samples increased during storage. The highest pH of 4.4 was recorded for control fruit on 20th day of storage whereas for the coated fruits on 30th day of storage was 4.0. Coatings reduced the increase in pH. The increase in pH is due to the organic acids which provide most of the hydrogen ions in tomatoes and normally decrease with ripening produce an increase in pH. The weight loss observed in control was due to the shrinkage of fruits by loss of moisture which was observed to a less extent in the coated fruits. The titratable acidity (TA) of the tomatoes decreased with maturity. The less pronounced decrease in titratable acidity in coated tomatoes indicates the effectiveness of coating films in reducing ethylene production, which accelerates the maturation of the fruit. Lycopene content of tomato increased during its ripening. There was a steady increase in the lycopene content of both the control and coated tomatoes but to a lesser extent in the 2.5% chitosan than 2%, 1%, 0.5% chitosan treatments. Overall based on physico-chemical data, it could be concluded that 2.5% chitosan resulted in better extension of the shelf life of tomato on storage.

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