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ORIGINAL ARTICLE

Effect of antimicrobial chitosan based films on the physicochemical properties of Tomato

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ABSTRACT
Edible coating and film is an attractive technology in the packaging of fruits and vegetables to reduce postharvest losses. Different sources are used for the preparation of films and coating materials with the incorporation of antimicrobial agents. In the present research, an antimicrobial assay of the different concentration of chitosan (0.5,1,1.5 & 2%) was carried out to check its efficacy for the prevention of certain microbes. Furthermore, the physicochemical properties of chitosan packed tomato were tested. Physiological loss in weight, total soluble solids, titratable acidity and pH of coated tomatoes have been investigated. Results indicated that chitosan improved the quality attributes in tomatoes significantly. Chitosan (CH) film packed tomato fruits showed reduced weight loss, deferred changes in titratable acidity and total soluble solids. The non-significant difference was found in pH of chitosan-coated tomatoes. From the current research, it can be expressed that chitosan packaging not only acts as antimicrobial packing but also enhance the shelf life of tomato fruits by maintaining their quality attributes.
Keywords: *Antimicrobial Packaging, Chitosan, Tomato, Physicochemical properties*

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INTRODUCTION

Postharvest losses in fruits is a severe issue in the world. It is due to poor maintenance in infrastructure, rapid loss occurs due to physicochemical and microbial deterioration during handling, transport, and storage. Tomato (*Lycopersicon esculentum*) is one of the most extensively eaten fresh fruit throughout the world. Nevertheless, its highly perishable nature which reduces its long term use after harvesting. To prevent the losses of this fruits and to preserve its quality attributes after harvesting, employment of appropriate preservative methods are necessary. Various kinds of preventive methods have been executed to reduce these losses. Edible coating, packaging with organic and biodegradable materials also have been tried and successfully applied by many researchers. Different materials can be used to make biodegradable packaging viz. polysaccharides, proteins, lipids or their combinations. Biopolymers are attracting in the search for both renewable and biodegradable materials. Among all, polysaccharides are the most commonly used. Particularly, chitin is a polysaccharide which is extracted from marine sources. Chitin is famous for its bioavailability as it ranks second in abundance after cellulose in nature. Besides, it is the compound that not only has the highest production rate but also is biodegradable in nature [9]. Chitosan is obtained from chitin after alkaline deacetylation. Being polycationic it has a high molecular weight (MW) and is a linear copolymer composed of β(1,4)- linked with N-acetyl-D-glucosamine units. Chitosan is well known for its film-forming properties. Moreover, is possesses beneficial characteristics such as antioxidant, antifungal, and antimicrobial nature. Also, it is a barrier to gases and water vapors which helps in improving the shelf life food [18]. It is believed that chitosan has outstanding resistance against different deteriorative causes such as biotic and abiotic stress [4]. Petriccione *et al* [10] studied the coating of strawberry fruits with chitosan. They revealed that chitosan coating increased the shelf life of the fruits by lowering the loss of moisture and delayed the changes in titratable acidity and rate of respiration. Chitosan mostly utilized as a food additive and material used for packaging which reduces the microbial count as well as enhances the quality of food [17]. Ansorena *et al.,* [1] studied the chitosan-coated broccoli and stated that the application of coating reduced the microbial growth significantly on samples. Additionally, it preserved the quality characteristics of food and helped to improve its shelf life. In this research, the desired concentration of chitosan is used to make the films as it is selected from the previous studies. Tomatoes were packed into the films and their physicochemical attributes are checked to examine the efficacy of chitosan in extending the shelf life of said fruits.

MATERIAL AND METHODS

Chitosan obtained by extraction from shrimp shells waste in the previous studies. Antimicrobial activity of extracted chitosan concentrations (0.5,1, 1.5 and 2 %) was checked.

Antimicrobial Analysis

Agar well diffusion method was carried out as reported by Sena *et al*., [15] .Antimicrobial activity was assayed by the method of agar well diffusion. Nutrient agar plates were swabbed (sterile cotton swabs) with 8 hours of an old - broth culture of *Staphylococcus aureus* and *Pseudomonas aeroginosa***.** Wells (10mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. The stock solution of chitosan was prepared at a concentration of 0.5, 1, 1.5, and 2mg/ml in 1 % acetic acid. These different concentrations were added into the wells and allowed to diffuse at 4^0C for 10 min. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zone (mm) was measured. Four replications were maintained and the average values were recorded.

Preparation of films.

For the preparation of films, 2 % chitosan was selected from the previous study as they were possessing good mechanical and barrier properties. Then they were further used to pack the tomatoes. Tomatoes **(***Lycopersicon esculentum*) were procured from the local market. Storage study of control samples as well as chitosan film covered tomatoes was carried out up to 8 days at room temperature. PLW, TSS, Titratable acidity, pH of control samples and chitosan film covered samples were carried out on an alternate day.

Physiological loss in weight (PLW)

The initial and final weight of sample was recorded and physiological loss in weight was calculated according to Restrepo *et al* [13].

Formula:

$$\text{PLW}= \frac{\text{Initial weight} - \text{Final weight Initial weight}}{\text{Initial weight}} \text{ X } 100$$

pH

The pH of the sample was recorded using a pH meter [2].

Total soluble Solid (TSS)

Total soluble solids were measured by a refractometer [2].

Titratable Acidity (TA)

Titratable acidity was determined according to the method of AOAC [2]. Each sample was treated with 0.1N NaOH solution using a titration kit, where 3 to 5 drops of phenolphthalein indicator were used. Volume of alkaline solution used for the experiment was recorded and the titratable acidity was calculated.

$$\text{TA}=\frac{\text{Titre reading X Eq.weight of acid}}{\text{Wt.of sample X 1000 X DR}} \text{ X } 100$$

$$\text{D.R.} = \frac{\text{Volume of sample}}{\text{Made up volume}}$$

Eq. wt. of citric acid=64

RESULTS AND DISCUSSIONS

Antimocrobial activity of chitosan

Experimental studies showed that tested concentrations (0.5, 1, 1.5 & 2 %) of chitosan exhibits good antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The results are in agreement with Rejane *et al.,* [12] who said that 1% of chitosan has an inhibitory effect against *S. Aureus*. They have shown that the most suitable antimicrobial function of chitosan includes the involvement of the charged group in the polymer backbone and their ionic contacts with bacterial wall constituents. This reaction signals the emergence of peptidoglycan hydrolysis in the membrane of the microorganism, contributing to leakage of intracellular electrolytes, leading to the death of the microorganism. Likewise, chitosan derivatives have inhibitory activity against *P.aeruginosa.* This research was illustrated by Liu *et al.* [8]. The inhibition of bacterial adhesion and biofilm development by anionic chitosan and cationic

chitosan was evaluated against *P. aeruginosa*. The study found that chitosan derivatives were effective in inhibiting the biofilm formation of *P. aeruginosa* and proposed chitosan as a potential alternative for the prevention of bacteriological pathogen in the food trade.
The zone of inhibition studies was carried out and diameter measured for the said organisms is reported in Table 1.

Table 1. Antimicrobial activity of different concentrations of chitosan

Test organism	Zone of inhibition in mm			
	0.5%	1%	1.5%	2%
S. aureus	20	21	21	22
P. aeruginosa	18	20	21	21.5

The above table demonstrates that the increasing concentration of chitosan showed the maximum inhibition to mentioned microbes which displayed the antimicrobial activity of chitosan. Van *et al* [16] got the similar outcomes who worked on the antimicrobial activity of chitosan methodically. They prepared chitosan solutions at diluted concentrations and in spray form, which applied to apples and bananas. It significantly prolonged the shelf life of the fruits and constrained the microbiological population and their growth. The evaluation informed that chitosan possesses strong antimicrobial behavior against pathogenic and spoilage microorganisms, including fungi, and both Gram-positive and Gram-negative bacteria.
From the previous studies it was proved that chitosan film with 2% concentration had better mechanical properties and high antibacterial activity compared to other films. Hence the concentration of 2% was selected for storage study. For this study tomatoes were selected and packed in chitosan film and stored at room temperature. During the storage study different parameters like weight loss, acidity, pH, TSS were calculated on alternate day and results obtained are given in Table 2.

Physico-chemical changes in tomato during storage at room temperature

Table 2. Changes in PLW and TSS during storage study of tomato at room temperature

Storage days	PLW (%)		TSS (%)	
	Control	**Chitosan packed**	**Control**	**Chitosan packed**
0	0	0	4.50±0.17e	4.32±0.23d
2	20.08±0.09d	6.27±0.04d	5.40±0.11d	4.72±0.04c
4	22.45±0.15c	7.51±0.08c	5.89±0.04c	4.82±0.01c
6	50.38±0.14b	8.39±0.08b	6.46±0.04bb	5.11±0.06b
8	54.26±0.28a	10.27±0.18a	6.75±0.22a	5.27±0.04a
LSD	0.24	0.22	0.20	0.16
CV	0.50	1.97	2.31	2.28

The data in table 2 showed significant difference in control and chitosan packed tomato. Greater weight loss was found in control samples on the 8th day of storage with the level of 54.26±0.28 from 20.08±0.08 whereas chitosan coated tomatoes showed the PLW of 10.27±0.18 which suggests that chitosan packaging significantly reduce the PLW in tomatoes. The results are similar to the outcomes given by Leak *et al* [7] who told that Chitosan lessened weight loss in ambient stored tomato fruits. Several experiments have shown that chitosan is more active in preventing weight loss in banana and mangoes [6] and strawberries [14] than other polymers such as starch and alternatives.
Moreover, control tomatoes showed more increase i. e. from 4.50±0.17 to 6.75±0.22 in total soluble solids than in chitosan film packed tomatoes in which the change seems from 4.32±0.23 to 5.27±0.04. This means that the chitosan delayed the changes in TSS of tomatoes during the storage. These results are in agreement with Eshetu *et al* [3]. Although there is significant difference among fruits before application of the coating (at zero day), there is faster increasing trends for control treatments than coated fruits in terms of TSS.
Fig 1 and fig 2 showed significant decrease in pH and increase in acidity in control samples and chitosan packed tomatoes. The graphical view finds that the chitosan packed tomatoes showed delay increase in titratable acidity and decrease in pH. These results are more or less similar to Ghaouth *et al* [5]. They coated tomatoes with chitosan solutions which reduced its respiration rate and ethylene production, with greater effect at 2% than 1% chitosan. Coating increased the internal CO_2, and decreased the internal O_2 levels of the tomatoes. Chitosan-coated tomatoes were firmer, higher in titratable acidity, less decayed, and exhibited less red pigmentation than the control fruit at the end of storage. The same results were

observed in the Raffo *et al*. [11] study, which shows that acidity decreased with maturation and increased with a high percentage of fruit sugar content in cherry tomatoes. The rise in pH indicates that most of the hydrogen ions in tomatoes are formed by organic acids, which usually decrease with maturation and cause an increase in pH.

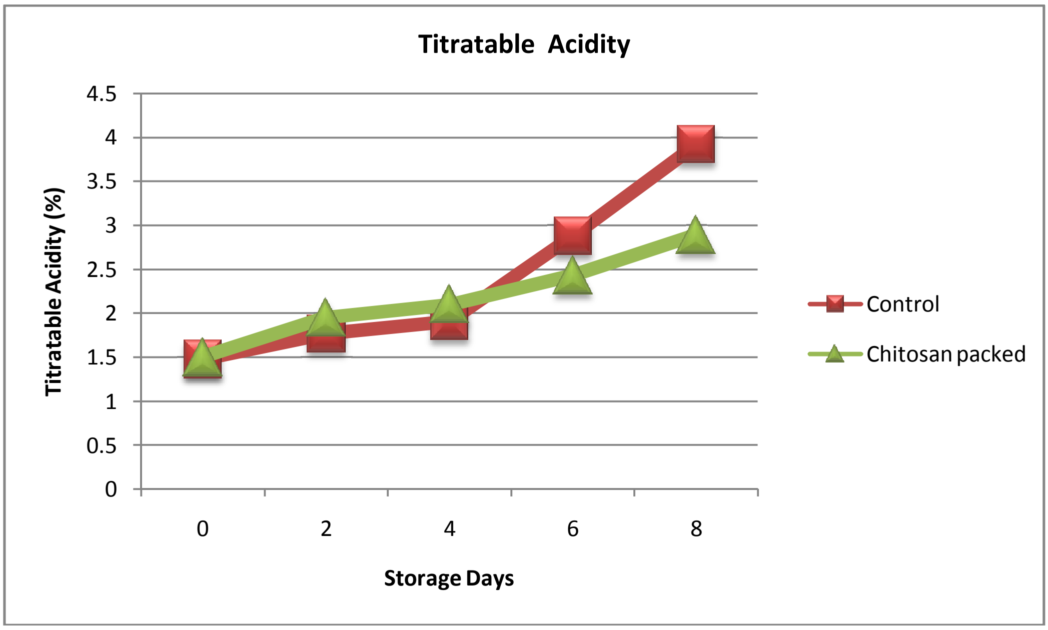

Figure 1. Changes in Titratable Acidity during storage study of tomato at room temperature

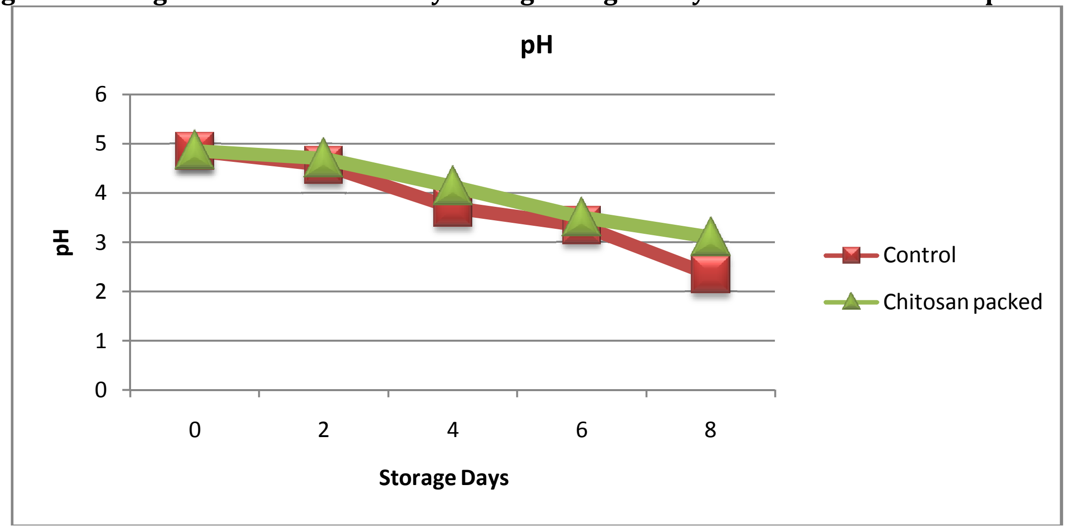

Figure 2. Changes in pH during storage study of tomato at room temperature

In the case of the control sample as the storage, period increased acidity decreased and TSS, and pH increased. But in the case of chitosan packed film, as the storage period, increased acidity decreased slightly and TSS and pH increased slightly. Zhelyazkov *et al.* [19] investigated the possible use of chitosan coating on fresh-cut apples in their research. Manually sliced apples were treated with solutions of 1, 2.5, 5 and 10 g.kg-1 chitosan in acetic acid and then stored at 4°C for 17 days. They established parallel results. Coating with chitosan hindered water loss and the fall in sensory quality, increasing the soluble solid content and decreasing titratable acidity. They proved that applying a chitosan coating preserved effectively the quality and extended the shelf life of fresh-cut apples. Chitosan packed tomatoes did not show any deterioration in tomatoes while the control sample showed deterioration. Its shelf life extended up to 8 days at room temperature.

Visual appearance of tomatoes on the 8th day of storage at room temperature

From the fig. 4 it can be clearly seen that tomatoes coated with chitosan has shown glossy appearance while control tomato was looking dull, bruised and ripen. Chitosan coating helped to increase the glossiness of tomatoes even after 7 days.

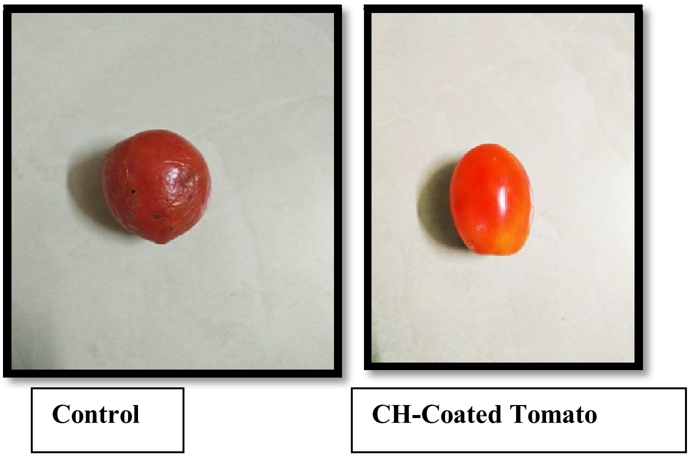

Fig 4 Visual appearance of tomatoes

CONCLUSION

In this study different concentration of chitosan (0.5, 1,1.5& 2%) optimized to check the antimicrobial activity. Antimicrobial activity of chitosan proved that concentration at 2% inhibits the development of microbes such as *Pseudomonas aeruginosa and Staphylococcus aureus*. Physiological loss in weight, Total Soluble Solid, acidity and pH of control and chitosan packed tomatoes were checked. It was found that chitosan packed tomatoes showed a slight increase in physiological loss in weight, Total soluble solids and Titratable acidity while a slight decrease in pH as compared to the control sample. It shows that it extends the shelf life of tomatoes up to 8 days at room temperature. It has convincingly been proved that chitosan films exhibit good antimicrobial activity which can help to extend the shelf life of tomatoes. At 2% chitosan concentration the films showed the best results in not only in terms of physico-chemical attributes but also in terms of glossiness. Hence, it is going to be no surprise if we witness the widespread use of chitosan films in tomorrow's food packaging.

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