Effect of chitosan-based coating enriched with starch nanocrystals and orange oil on the nutritional properties of tomatoes

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Abstract
Nano-based chitosan solution enriched with orange oil was used for the surface coating of tomatoes. The nutritional properties of the coated tomatoes stored at room temperature (30 ± 2ºC) for 15 days were investigated. Tomatoes coated with the modified coating solution showed a weight loss of less than 7 ± 0.4% and observed an enhanced pH value as compared to that of uncoated tomatoes. Investigation on total sugar content, lycopene content, anthocyanin content, vitamin C content, Phenol and flavonoid content revealed that tomatoes coated with orange oil-enriched solution maintained its quality similar to that of fresh tomatoes.

Similarly, the coated tomatoes retained its antioxidant activity as that of fresh tomatoes i.e. tomatoes coated with CGO-SNCs film solution showed maximum radical scavenging activity (40 ± 0.5% at 100 µg/mL) with an IC_{50} value of 174.4 µg/mL. This result indicates that the coated tomatoes maintained their freshness and safety during the entire storage period.

Keywords: Chitosan, Coated tomatoes, Orange Oil, Starch Nanocrystals, Antioxidant property.

Introduction

Food loss and waste are global issues occurring due to limited harvesting capabilities, inadequate storage, problems in transportation and processing, especially packages. This results in a challenge to food security, food safety, the economy, and, ultimately environmental sustainability. Improper packaging and non-degradable packaging materials result in the development of the gross amount of food waste and packaging waste respectively. Proper packaging of food items helps to face these problems to a more considerable extent.

Usually, all of the food items that we buy and consume are packaged in some way or another. The main functions of food packaging and coating are to protect and preserve the food from physical damage and from microorganisms, insects, pests etc. to maintain quality and safety and also to reduce food waste. Even though packaging helps to avoid food waste to some extent, the increased use of synthetic packaging films has led to a severe ecological problem due to their total non-biodegradability. There can be no doubt that modern food packaging materials and technologies fulfill these functions and packaging plays a key role in helping to provide a safe and nutritious food supply.

The use of biodegradable polymers for food packaging and the advent of nanotechnology, which involves the development and use of materials in size range of up to about 100 nm in one or more dimensions, has paved a new way for the development of advanced materials with improved properties. A number of world's largest food companies have been reported to be actively exploring the potential of nanomaterials for use in food or food packaging[7]. The present study embodies the effect of modified chitosan-based packaging films incorporated with starch nanocrystals and essential oils on the nutritional quality and safety of tomatoes.

Material and Methods

Materials: All reagents used for this study are of analytical grade. Trichloroacetic acid (TCA), iron (II) sulfate heptahydrate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) etc. were purchased from M/s Merck (Germany) while glacial acetic acid, glycerol, sodium phosphate monobasic and sodium phosphate dibasic were purchased from M/s SRL Pvt. Ltd. Folin and ciocalteu's phenol reagent, EDTA, streptomycin, ascorbic acid and all other microbiological media were obtained from M/s HiMedia Laboratories Pvt. Ltd., Mumbai, India. The tomatoes (*Solanum lycopersicum*) for this study were selected from an organic farm, Attingal, Thiruvananthapuram.

Chitosan for the study was obtained from M/S Nitta Gelatin India Ltd., Kerala and native sago was obtained from a local grocery store. 3T3 cell line for the study was obtained from Inter-University Centre for Genomics and Gene Technology, University of Kerala, India.

Formulation of nano-based chitosan solution enriched with orange oil: Chitosan-glycerol (CG) films incorporated with starch nanocrystals and orange oil (CGO-SNCs) were prepared by dissolving crab shell chitosan (400 KDa, 76% deacetylated) powder in 2% (v/v) glacial acetic acid followed by the addition of 1% glycerol, 0.1% sodium alginate and 1% lactic acid. The dissolved chitosan solution was then filtered through a Whatmann no. 1 filter paper and allowed to stabilize at room temperature (30 ± 2 °C) for 12 h. The solutions were mixed with 500 µL of essential oil (orange oil) and 1 mL of starch nanocrystals (S-NCs) (1mg/1mL)[17].

SEM analysis of modified chitosan solution: The microstructure of the film-forming solution was observed using a Scanning electron microscopy (Nova Nanosem 450) at a voltage of 5 kV. The solution was coated on a glass slide (1 x 1 cm^2) and allowed to dry at 40 ˚C. The film samples so formed were sputtered with a thin layer of gold in an ion sputter coater (QUORUM- Q150R ES, UK) and placed into the SEM chamber to observe the surface of the films.

Toxicity study of film-forming solution incorporated with essential oils

Cell culture: 3T3 fibroblast normal cell lines maintained at Inter-University Centre for Genomics and Gene Technology, University of Kerala, India, were used for the study. 3T3 is a mouse embryonic fibroblast cell line. The 3T3 cells were cultured in DMEM medium supplemented with FBS, antibiotic: antimycotic (1X) and 5% CO_2 at 37 ˚C. Cells were passaged regularly and subcultured to 80% confluence before the experiments. Approximately 1000 cells were treated with different samples of modified film-forming solutions for 24, 48 and 72 h in 96-well plates at different concentrations. Cell viability was determined using MTT assay. About 1mg/mL of starch nanocrystals were taken as the stock.

CGO-SNCs coating solution was divided into eleven samples (v/v):

Sample I: CG (1000 µL)
Sample II: CG (999.02 µL) + EO (0.49 µL) + SNCs (0.49 µL)
Sample III: CG (998.05 µL) + EO (0.98µL) + SNCs (0.98 µL)
Sample IV: CG (996.09 µL) + EO (1.96 µL) + SNCs (1.96 µL)
Sample V: CG (992.19 µL) + EO (3.91 µL) + SNCs (3.91 µL)
Sample VI: CG (984.38 µL) + EO (7.81 µL) + SNCs (7.81 µL)
Sample VII: CG (968.74 µL) + EO (15.63 µL) + SNCs (15.63 µL)
Sample VIII: CG (937.5 µL) + EO (31.25 µL) + SNCs (31.25 µL)
Sample IX: CG (875 µL) + EO (62.5 µL) + SNCs (62.5 µL)
Sample X: CG (750 µL) + EO (125 µL) + SNCs (125 µL)
Sample XI: CG (500 µL) + EO (250 µL) + SNCs (250 µL)

MTT assay: For MTT assay, culture media from titer plates were removed after 24 h incubation; MTT (0.5 mg mL^{-1} prepared in fresh basal media) was added to the cells and incubated for 4 h. After incubation, DMSO was added to the wells and absorbance was measured at 570 nm in a microplate reader (M/s Multiskan Go).

Coating of tomatoes: Herein the randomly selected tomatoes were taken and the dipping method has been used for coating samples. The method of Schneller et al[31] was followed, in which a membranous film was formed over the sample surface by directly dipping the sample into the CG solution and CG solution incorporated with orange oil and starch nanocrystals (CGO-SNCs) followed by air-drying of dipped tomatoes. This dipping technique forms a thin coating layer on the surface of the tomatoes. Tomato, which was used as the control, does not have any coating and named as 'uncoated tomato.' Another one, which was coated using the CG coating solution, was named as 'coated CG' while tomatoes coated with CGO-SNCs solution were named as 'coated CGO-SNCs.' All of these tomatoes were placed at room temperature (30 ± 2 ˚C) for 15 days.

Biochemical and physical evaluation of coated tomatoes: The tomato samples were evaluated for their physiological and biochemical changes after 15 days of storage at room temperature (30 ± 2 ˚C).

Evaluation of weight loss and pH: For evaluating the weight loss of tomatoes, the weight of tomatoes (uncoated, coated CG and coated CGO-SNCs) from each set was calculated at repeated intervals of 5 days. The weight loss was determined by following eq. 1:

$$\text{Weight loss (\%)} = (W_1\text{-}W_2/W_1) \times 100 \quad (1)$$

where W_1 is the initial weight of the samples and W_2 is the weight of the sample at each storage period. After 15 days of storage, the pH of each sample (Fresh, uncoated, coated CG and coated CGO-SNCs) was determined using pH meter.

Determination of total soluble sugar content: The total soluble sugar content of tomatoes (fresh, uncoated, coated CG and coated CGO-SNCs) was determined by using anthrone reagent. Herein the total soluble sugar was extracted using Omokolo, Tsala and Djocgoue[27] method with slight modifications. In this method, 40 mg of tomato fruit tissues (fresh, uncoated, coated CG and coated CGO-SNCs) were taken and mixed with 5 mL of ethanol (80%) in a hot water bath (70 ˚C). The resulting extracts were centrifuged (1000 rpm) for 15 min and the supernatant obtained was transferred to a beaker. The extracts were concentrated by heating to 1/5th of its volume. The resulting aqueous phase was centrifuged (10000 rpm) for 10 min and the clear upper phase was collected and used for the determination of soluble sugars.

The sugar so obtained was determined by following the method of McCready et al.[26] Anthrone reagent for the study was prepared by diluting the reagent (200 mg) in 100 mL concentrated H_2SO_4. Fructose (2 mg in 1 mL 80% ethanol) was taken as the standard. Different volumes (0.1, 0.2, 0.3, 0.4 and 0.5 mL) of each prepared sample of tomato (fresh, uncoated, coated CG and coated CGO-SNCs) and also the standard were taken in separate test tubes and each made up to 1 mL using 80% ethanol followed by the addition of anthrone reagent (2 mL) to all the test tubes. The samples were incubated in a boiling water bath (100 ˚C) for 10 min. After cooling, the absorbance of each sample was measured

at 620 nm using a UV-VIS spectrophotometer (Shimadzu UV-1700).

Determination of lycopene content: The lycopene content was determined by the method of Theeranat[33]. For determining lycopene content, first, a solvent system was made by mixing hexane: ethanol: acetone (2:1:1). This solvent mixture (8 mL) was added to an aqueous solution (0.1 mL) of each tomato samples (fresh, uncoated, coated CG and coated CGO-SNCs). The mixture was incubated for one h in the dark followed by the addition of 1 mL of deionized water to each sample. Then vortex the mixture and allow to stand for 10 min. OD was taken at 503 nm. The lycopene content was then calculated by the eq. 2:

$$\text{Lycopene content} = A_{503} \times 537 \times 8 \times 0.55 / 0.10 \times 172 \quad (2)$$

[A_{503}–molecular weight of lycopene, 8 mL - the volume of mixed solvent, 0.55 - volume ratio of the upper layer of the mixed solvent, 0.10 g - of tomato sample, 172 mM^{-1}-extinction coefficient for lycopene in hexane]

Determination of Vitamin C content of coated tomatoes: For determining the vitamin C content in tomatoes, 20 mL of tomato (fresh, uncoated, coated CG and coated CGO-SNCs) sample was mixed with 10% TCA (trichloroacetic acid). The volume was then made up to 100 mL using deionized water. From that mixture, 1 mL was taken and 3 mL deionized water was added. To that, 0.4 mL of Folin's reagent (1 mL Folin's reagent + 9 mL deionized water) was added. It was then incubated at room temperature (30 ± 2 °C) for 10 min. OD of samples was measured at 760 nm.

Determination of anthocyanin content: The anthocyanin content was measured by the method described by Wanger[38]. Tomato samples (fresh, uncoated, coated CG and coated CGO-SNCs) of 1g each were taken and mixed with 10 mL of 85% acidified methanol (85 mL methanol in 15 mL 0.1 M HCl). After thorough mixing, the reaction mixture was incubated at 4 °C for 24 h. The mixture was then centrifuged at 4000 rpm for 10 min. The absorbance of the supernatant was taken at 520 nm.

Determination of total phenolic content: The total phenol content in tomatoes was determined by the Folin-Ciocalteu method[13] with some modifications. Tomato samples (fresh, uncoated, coated CG and coated CGO-SNCs) were prepared by homogenization of 10 g of each sample in 10 mL deionized water. Different volumes (0.1, 0.2, 0.3, 0.4 and 0.5 mL) of each sample were taken and 0.5 mL of Folin's reagent (1: 10 dilutions; 1 mL Folin's reagent in 9 mL deionized water) was added to this sample and vortexed. The resulting mixture was then incubated for 5 min at room temperature (30 ± 2 °C) followed by the addition of 1 mL of 7.5% Na_2CO_3 and again incubate the assay tubes at room temperature (30 ± 2 °C) for 2 h. After 2 hours of reaction at ambient temperature (30 ± 2 °C), the absorbance was measured at 765 nm and the total phenol content was expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of fresh weight (FW).

Determination of total flavonoid content: The total flavonoid content was determined by means of aluminum chloride colorimetric method[44]. Quercetin (1 mg/mL) was taken as the standard. An aqueous solution of each sample (fresh, uncoated, coated CG and coated CG-SNCs tomatoes) was taken in different volumes (0.1, 0.2, 0.3, 0.4 and 0.5 mL) in separate test tubes. To each sample, 0.5 mL of 2% aluminum chloride was added and made up to 2 mL with methanol. The OD of each sample was taken at 420 nm. The total flavonoid content was expressed as milligrams of quercetin equivalent (QE) per 100 grams of fresh weight (FW). All analyses were performed in triplicate.

DPPH radical-scavenging activity of coated tomatoes: The antioxidant activity of the tomato samples (fresh, uncoated, coated CG and coated CGO-SNCs tomatoes) was evaluated using the modified protocol of DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to Byun et al[6]. Briefly, 10 mg of sample was taken and serially diluted and placed separately in test tubes containing 200 µL of DPPH solution. The tubes were then vortexed followed by incubation at 37 °C for 30 min in the dark (until stable absorption values were obtained). Optical densities (OD) of the samples were measured at 517 nm using a spectrophotometer (M/S Shimadzu - UV-1700, Japan). The activity was expressed in terms of IC_{50} (µg/mL). DPPH radical scavenging activity was calculated by using the following eq. 3:

$$\text{Radical scavenging activity (\%)} = A_{\text{control}} - A_{\text{sample}} / A_{\text{control}} \times 100 \quad (3)$$

where A_{sample} represents the absorbance of the sample solution and $A_{control}$ represents the absorbance of DPPH solution without the addition of the film-forming solution.

Preparation of protein samples from tomatoes: Total protein from tomato was extracted by homogenization using 27 mL of 5% SDS (sodium dodecyl sulfate) and centrifuged at 13,500 rpm for 2 min. Then the sample was incubated in a water bath at 85 °C for 1 hour. After 1 h of incubation, the sample was again centrifuged at 5000 rpm for 20 min. The supernatant was taken and the protein content in the tomato samples (Fresh, uncoated, coated CG and coated CGO-SNCs) was quantified by the method of Lowry et al.[24]

BSA (1 mg/mL) was taken as the standard. Different volumes (0.2, 0.4, 0.6, 0.8 and 1 mL) of each sample were taken and made up to 1 mL using 0.1 N NaOH followed by the addition of 5 mL of reagent 1 (2% sodium carbonate in 0.1 N NaOH + 1% of sodium potassium tartrate + 0.5% of copper sulfate) and incubated for 10 min. Then 0.5 mL of reagent 2 (1 mL of Folin's reagent +1 mL deionized water) was added to each of these samples. Incubate the sample for 30 min. OD was taken at 660 nm.

Microbiological changes - Determination of Total viable Count (TVC) of tomato samples: Microbiological counts of tomatoes were determined after 15 days of storage period. Microbiological characteristics of 100 g samples (fresh, uncoated, coated CG and coated CGO-SNCs) were obtained after homogenization in 9 mL 0.1% peptone water (Sisco Research Laboratories Pvt. Ltd). Other dilutions (10^{-1} to 10^{-5}) were prepared from the stock solution. The total count was determined using the pour plate method. Plates were incubated at 37 ˚C for 24 h.[18] The final three dilutions from each group were analyzed.

Statistical analysis: All experiments were conducted in triplicate (n =3). The results were expressed as mean values with standard deviation (SD).

Results and Discussion

Coating of tomatoes: Tomato fruit is one of the most widely consumed product items throughout the world. Tomatoes are rich in health-related compounds, as they are good sources of phenolics, flavonoid compounds, lycopene and ascorbic acid.[15] But it is an easily perishable fruit. Different studies have shown that the use of edible coating reduces tomato metabolism, thus increasing its shelf life. Herein the chitosan-based coating on tomatoes reduced the changes that affect most of the vital quality parameters involved in the tomatoes.

SEM analysis of coating solution: SEM images of the nano-based chitosan film enriched with orange oil (CGO-SNCs) and chitosan film are shown in fig. 1a and b. From the micrograph under magnification (50,000 X), the surface of CGO film in the absence of starch showed a randomly distributed microstructure. However, the surface of the CG films showed a smooth appearance.

Effect of CG, CGO-SNCs coating solution on cell viability of 3T3 cells: The time-course effects of different concentrations of the CG and CGO-SNCs on the viability of 3T3 normal cell lines for 24, 48 and 72 h are shown in fig. 2. The results showed that in the treated 3T3 cell line, all samples (CG and CGO-SNCs) did not evoke any significant decrease in the cell viability (cell death) compared to the control 3T3 cells. From these initial time-course experiments, it was established that an increase in cell death did not occur to its maximum even after 72 h of incubation. The cytotoxicity of modified coating solution over 3T3 cells was found not significant and hence no IC_{50} of any of the solutions was found.

Results showed that more than 97% of the cells were viable in CG and CGO-SNCs even at higher concentration in MTT assay. MTT results confirmed that all chitosan-based films demonstrated a lower rate of cell death. In an earlier report, it was noted that chitosan-based hydrogels modified with nanosilver do not affect negatively on epidermal cells. However, they inhibited the growth of *Escherichia coli*[5]. The chitosan film modified with aglyconegeniposidic acid demonstrated lower cytotoxicity; these results suggested that modified chitosan film with lower cytotoxicity may represent a promising and new type of edible coating for fruits and vegetables.

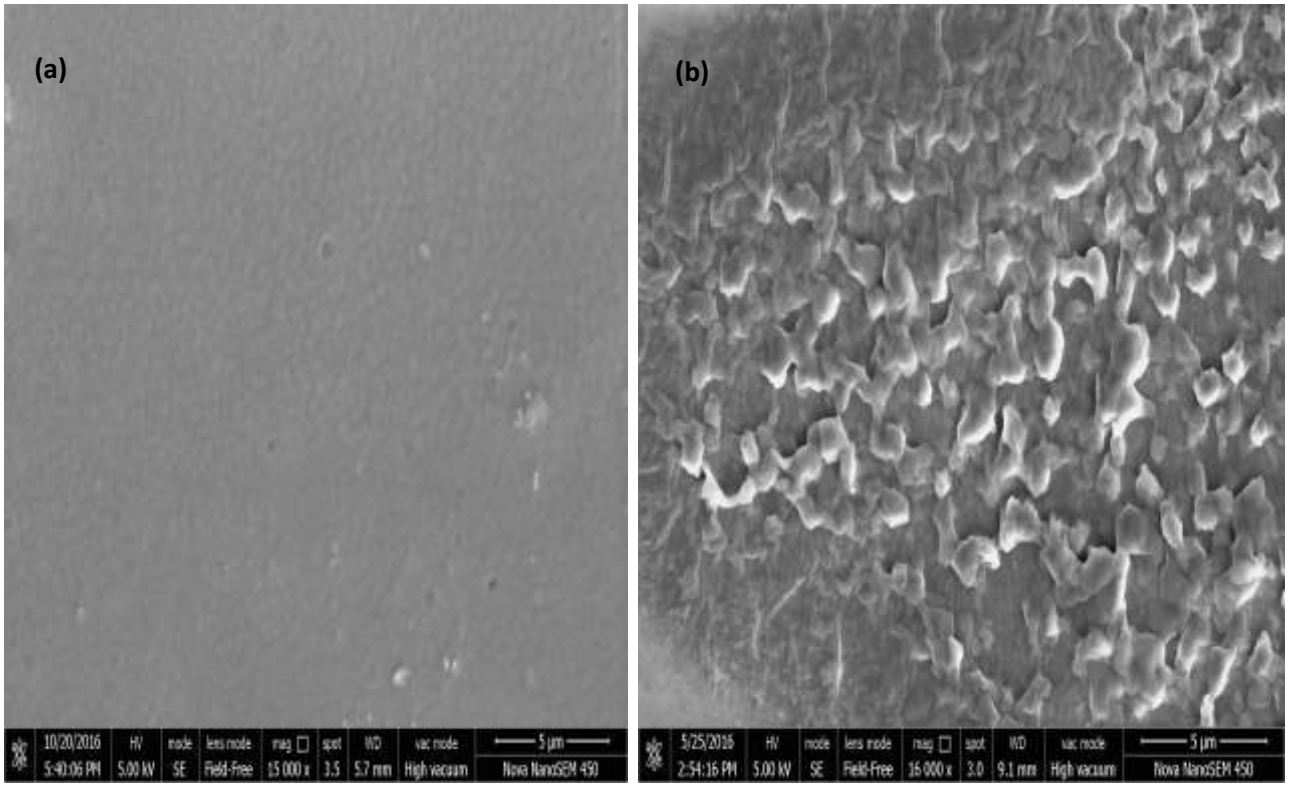

Fig. 1: SEM image of chitosan film (a) and chitosan film incorporated with starch nanocrystals and Orange oil

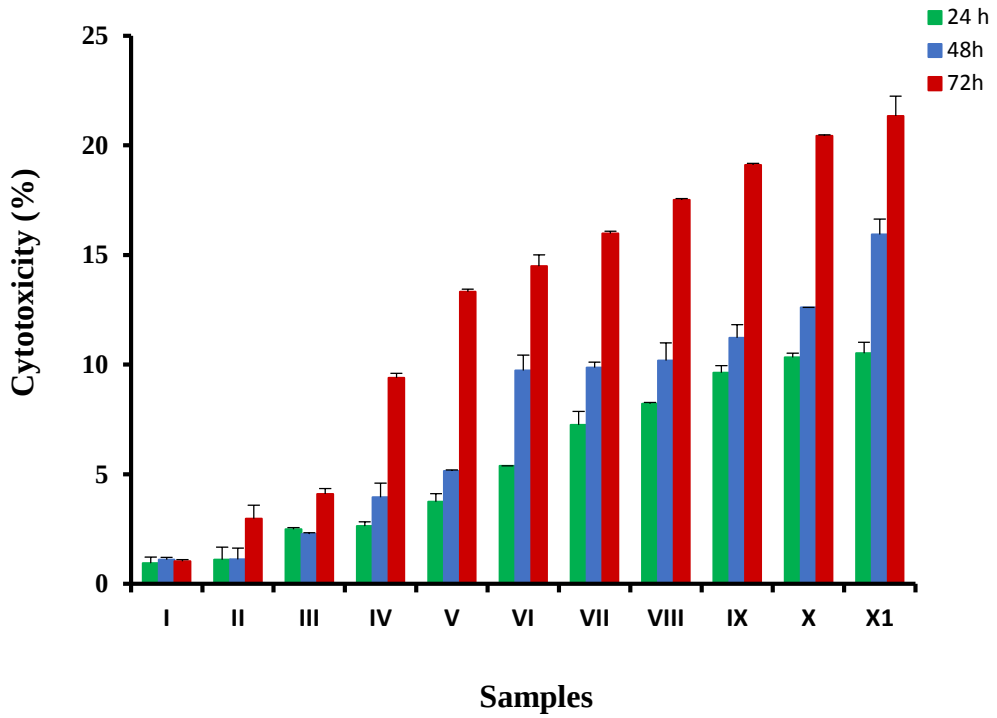

Fig. 2: Cytotoxicity of CGO-SNCs coating solutions against 3T3 cell line

Biochemical and physical evaluation of coated tomatoes

Evaluation of weight loss and pH: The weight loss increased significantly throughout the storage of tomatoes at room temperature. The highest weight loss was observed in the uncoated tomato sample, it reached 16.08 ± 0.5% at the end of the period.

According to the viewpoint of Bico et al[3], more than 4-6% (of the total fresh weight) weight loss was accompanied by visible wilting or wrinkling of the surface of the fruits and vegetables. For the coated (CG and CGO-SNCs) tomatoes, the weight loss was less than 7 ± 0.4% to that of uncoated ones which indicates that the coated tomatoes maintained the freshness during the entire storage period.

The weight loss of tomatoes was mainly due to the moisture loss and it may also vary significantly among coated and uncoated tomatoes even under similar storage conditions. This was in agreement with a previous report which explained the influence of transpiration and respiration in weight loss of fruits and vegetables[45]. Tomato is an easily perishable commodity with a low skin diffusion resistance[30] and high surface/volume ratio[9,39], which promotes rapid water loss both from the fruit and stem[29]. Moisture loss and gaseous exchange from fruits and vegetables are usually controlled by the epidermal layers provided with the guard cells and stomata.

Chitosan and essential oil-based coatings create a semi-permeable film which acts as a physical barrier that regulates gas exchange and reduces the transpiration rate which is generally determined by the gradient of water vapor pressure between the fruit and the surrounding air[2]. This effect on tomatoes is similar to that obtained with other edible coatings.[10,12,25,30,43]

From the pH study, it has been revealed that the pH of the samples under all coating conditions decreased as the storage time increased. Samples without chitosan coating showed the largest pH drop, probably caused by the growth of lactic acid and other aerobic bacteria that are present in easily perishable tomatoes. According to the Centre for Food Safety and Applied Nutrition at the Food and Drug Administration, fresh tomatoes fall into a pH range of 4.3 - 4.9. The acidity of fresh tomatoes is mainly associated with their degree of ripeness. Extreme values of the acidity are not suitable for fresh fruits. A very good correlation has been obtained between the growth of the spoilage flora and the decrease in pH values of various tomato samples stored at room temperature (30 ±2 $^{\circ}$C).

Coated tomatoes (CG and CGO-SNCs) showed smaller pH (Table 1) drop than the control samples (pH-4.4) because chitosan coating may create a significantly anaerobic environment that prevented the growth of aerobic spoilage organisms which are responsible for the quality changes and the spoilage of foods. Moreover, the antimicrobial activity of chitosan and orange oil prevented the growth of microbes and thereby helped to prevent the fall of pH.

Determination of total soluble sugar content: The total sugar of the fruit is considered to be one of the basic criteria to evaluate fruit ripening. The total sugar content (TSC) increase during the storage period can be due to the dehydration and decomposition of organic acids (used as an energy source) in the fruit[36]. Herein the carbohydrates are

dehydrated with concentrated sulfuric acid to form furfural which condenses with anthrone to form a green-colored complex. The total soluble sugar content of tomato fruits, especially uncoated tomatoes increased over the 15 days of storage. This increase in sugar content could be attributed to the breakdown of starch to sugar[1] during the ripening process. In tomatoes the major sugar is fructose.

Table 1
pH of uncoated, CG coated and CGO-SNCs coated tomatoes after 15 days

Sample	pH (room temperature)
Fresh tomato	4.4
Uncoated tomato	3.3
CG coated tomato	3.9
CGO-SNCs coated tomato	4

Normally, fruits contain starch, pectic material, carbohydrates such as sucrose, fructose and glucose. Each of these sugars highly increases during ripening of fruit, because all of the starch and cell wall polysaccharides get fully hydrolyzed[8]. The coating can reduce the increase in sugar content by reducing the respiration rate. One gram of uncoated tomato fruit (100 µL) showed the presence of 1.04 mg/mL of TSC whereas the coated CG and CGO-SNCs fruit (1g) showed 0.66 mg/mL and 0.42 mg/mL of total soluble sugar respectively. The lowest TSC (0.31 mg/mL) value was observed for fresh tomato fruit. Here, fructose was used as the standard.

Determination of lycopene content: Lycopene is the major carotenoid pigment characterized by a symmetrical and acyclic structure containing 11 conjugated double bonds. This structure is responsible for the red-orange color of fruits such as tomatoes, watermelon and red grapefruit[35]. Studies carried out in Great Britain revealed that the lycopene content of tomatoes with intensive red color was 50 mg/kg; the yellow variety of tomatoes was characterized by a 10-fold lower content of lycopene i.e. 5 mg/kg[19]. The lycopene content of uncoated and coated tomatoes is given in table 2.

Table 2
Lycopene content of different tomato samples

Sample	Lycopene content on the 15th day of storage (mg/100g)
Fresh tomato	11.625
Uncoated tomato	15.496
CG coated tomato	14.014
CGO-SNCs coated tomato	12.228

Previous reports reveal that the lycopene content of tomatoes was in the range of 1.86-14.62 mg/100 g of its fresh weight[15]. Therefore, the difference in the lycopene content could be attributed to the retardation of the fruit maturity process caused by the combination of the temperature of storage and the edible coating applied to it. The higher level of the lycopene indicates an enhanced ripening in the fruit which could be observed more in the uncoated control fruit (15.496 mg/100 g) than in the fresh (11.625 mg/100g) and coated fruit. During the 15th day of storage, a reduced lycopene content was observed in both CGO-SNCs coated tomato (12.228 mg/100g) and 14.014 mg/100 g in CG coated tomato.

Determination of Vitamin C content: The vitamin C content of fresh and coated tomatoes retained almost similar values during the storage period while uncoated tomatoes showed a slight initial enhancement in the content of vitamin C (after 5-6 days) as compared to that of fresh tomatoes followed by a slight decrease of vitamin C (after 15 days) i.e. it showed a reduction of 28 ± 0.7% and finally it reached its degradation stage (almost 50 days). The reduction in the loss of ascorbic acid of coated tomatoes may be due to the low oxygen permeability of the chitosan coating, which lowered the activity of the enzymes and prevented the oxidation of vitamin C[10].

Determination of total anthocyanin content: Anthocyanins are the most prevalent class of purple, red and blue plant pigments which come under the flavonoid group. At harvest, the total anthocyanin content is lower in the tomatoes and later increased significantly during storage. No significant changes in the total anthocyanin content were observed in coated fruit over the 15 days of storage as compared to that of fresh fruit. On 15 days of storage, it was found that uncoated tomatoes exhibited an increase in the total anthocyanin content (0.24 mg/g) due to normal ripening process and with the increase in storage period, it exhibited a slight decrease in anthocyanin content due to the spoilage of tomato whereas the CG (0.146 mg/g) and CGO-SNCs (0.118 mg/g) coated tomatoes showed only 8.6% and 5.8% increase in anthocyanin content. This result concludes that chitosan coating helped to maintain the level of total anthocyanin content, almost similar to that of the fresh fruit.

Among the analyzed cultivars, different types of anthocyanins have been identified in tomatoes; the most highly represented included petunidin, delphinidin and malvidin[4]. The concentration and distribution of different anthocyanins are mainly restricted on the outer surface (skin) of tomatoes and this influences the color of tomatoes. Anthocyanin accumulation during storage is attributed to normal tomato ripening. Here uncoated tomato will undergo normal ripening stages while in the case of coated tomatoes, most of its metabolic activity gets arrested due to coating.

Wong et al[40] suggested that the edible coating formed a gas barrier, probably due to the dense structure of the coating and resulted in a modification of the internal atmosphere of coated samples. This seemed to delay anthocyanin synthesis and degradation in tomato samples.

Determination of total phenolic content: Polyphenols, widespread constituents of fruits and vegetables[14] are considered as the most abundant antioxidants in the diet. However, they are susceptible to degradation during storage. In the present study, comparatively higher total phenolic content values were observed for both coated tomatoes (CG and CGO-SNCs) i.e. 11.76 mg (GAE)/g for CGO-SNCs treated tomato sample and 10.92 mg (GAE)/g of TPC for CG treated sample respectively (Fig. 3.). The uncoated tomatoes showed a lower total phenolic content of 8.46 mg (GAE)/g. In fact, chitosan treatment influenced the total phenolic content during post-harvest life, improving the nutraceutical properties of tomatoes.

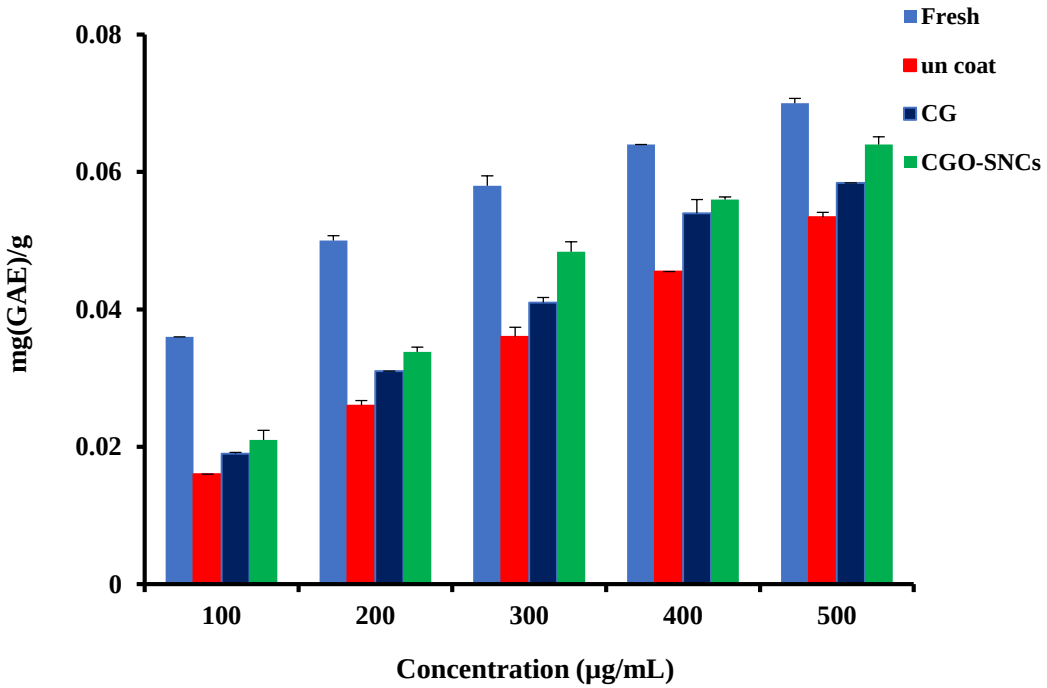

Fig. 3: Total Phenolic content of coated and un coated tomatoes

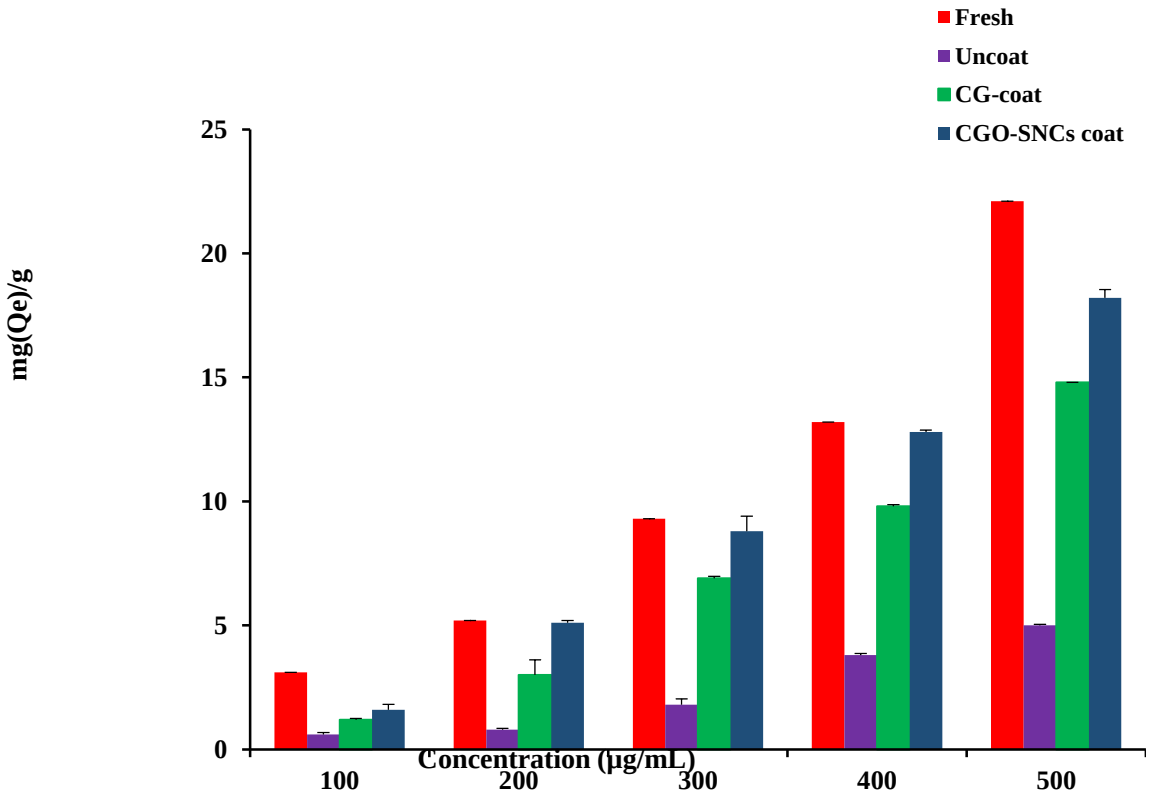

Fig. 4: Total flavonoid content of coated and uncoated tomatoes

Determination of total flavonoid content: The genotype influences the total flavonoid content in the tomatoes. Naturally, under storage conditions, the total flavonoid content of tomatoes gets decreased. But in the present study, the coating of tomatoes using CG and CGO-SNCs solution helps to maintain of flavonoid content similar to that of fresh tomato. Flavonoids have an essential role in the human diet, reducing oxidative stress in biological systems due to their antioxidant capacities[23]. In the case of uncoated tomatoes, it showed a total flavonoid content of 49 mg/g whereas tomatoes coated with CGO and CG solution showed 134 and 126 mg/g of total flavonoid content respectively (Fig. 4).

The fresh tomatoes showed 145 mg/g of flavonoid content. Even though an increased content of anthocyanin (a type flavonoid) was noted in uncoated tomatoes, a decrease in other major flavonoids such as rutin[28], naringenin[16,28,41] and chalconaringenin[20,22,32] and some minor flavonoids such as kaempferol 3-rutinoside[16] and naringenin 7-glucoside[20] resulted in an overall reduction of the flavonoid content. Major flavonoids of the tomatoes are mainly restricted to its skin rather than other parts of tomatoes[11]. This report is in agreement with our work where the uncoated tomatoes on storage at room temperature result in wrinkling and breakage of the outer surface of tomatoes followed by the loss of major flavonoids. The coated tomatoes retained the flavonoid content by preventing the normal ripening process.

Determination of antioxidant activity: The antioxidant activities of tomatoes (coated and uncoated) were measured by the DPPH assay (Fig. 5). Usually, during storage conditions, a decrease in the antioxidant activity was noted for tomatoes. Herein, for uncoated tomatoes, slightly higher antioxidant values were found at its initial storage period and later, the antioxidant activity was found to be decreased (after 15 days). The coated tomatoes retained similar antioxidant activity as that of fresh tomatoes. Here tomatoes coated with CGO-SNCs film solution showed maximum radical scavenging activity (40 ± 0.5% at 100 µg/mL) with an IC_{50} value of 174.4 µg/mL and CG coated tomatoes showed a radical scavenging activity of 37.6 ± 1.2% at 100 µg/mL.

The uncoated tomatoes showed an activity of 35 ± 0.04% at 100 µg/mL. The results revealed that the coating helped to maintain the natural antioxidant activity of tomatoes. Moreover, the enhanced antioxidant capacity of CGO-SNCs coated tomatoes can be attributed to synergistic and additive effects between different phytochemicals[23,34] and also chitosan nanomaterial helps to maintain the stability of antioxidants[21].

Determination of protein content: The protein content of the tomatoes was determined by Lowry's method.[24] The protein content in harvested tomato is 7.9 mg/g of tomato. The uncoated tomatoes gave 3.2 mg/g of protein and tomatoes coated with CGO-SNCs gave 6.2 mg/g of protein. The protein content decreased with increase in storage period. Here tomatoes coated with CG and CGO-SNCs showed a slight decrease in protein content.

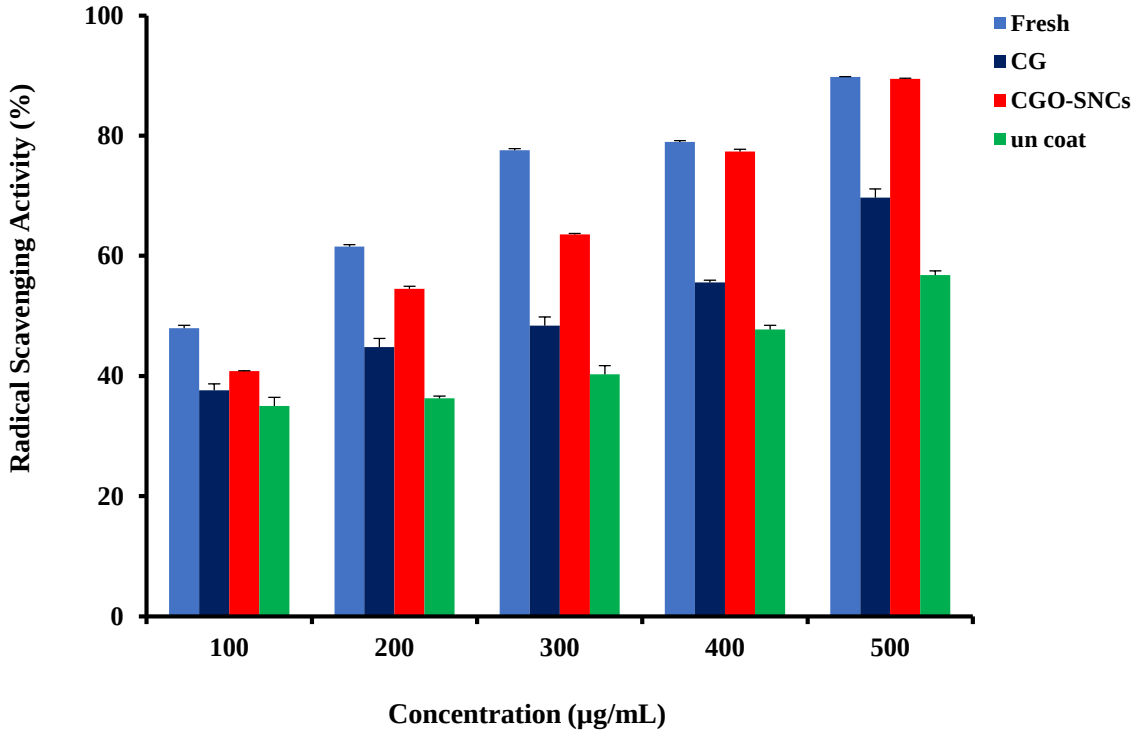

Fig. 5: Radical scavenging activity of (a) coated and uncoated tomatoes and standard graph of DPPH (Ascorbic acid)

Table 3
Total viable content of fresh, uncoated CG coated and CGO-SNCs coated tomatoes

S. N.	Samples	Microbial count	
		Dilution	CFU/mL
1	Fresh (Tomato)	10^{-3}	2.5×10^5
		10^{-4}	1.4×10^6
		10^{-5}	9×10^6
2	Uncoated (Tomato)	10^{-3}	8.6×10^5
		10^{-4}	3.3×10^6
		10^{-5}	2×10^7
3	CG Coated (Tomato)	10^{-3}	1.6×10^5
		10^{-4}	1.2×10^6
		10^{-5}	6×10^6
4	CGO-SNCs coated (Tomato)	10^{-3}	1×10^5
		10^{-4}	2×10^5
		10^{-5}	0

The highest reduction in protein content was observed in the uncoated tomato samples. The uncoated tomato showed a 53.03% reduction in total protein content and the coated tomato showed only a 14.93% reduction in protein content when compared with the fresh control.

Microbiological changes

Total viable count of tomatoes: Numerous coating materials along with different storage methods can be used to enhance the shelf life of perishable fruits and vegetables. Here also the key steps needed for fruit storage are to inhibit the growth of pathogenic microorganisms and preserve fruits and vegetables from microbial spoilage. Microbial count analysis of coated and uncoated tomatoes after 15 days of storage at room temperature revealed that microbial growth was more rapid in uncoated samples with a total viable count of 8.6×10^5 CFU/mL for 10^{-3} dilution (Table 3).

In this study, the quality of tomatoes was unacceptable when the microbial count exceeded 10^6 CFU/g[37] and the sample is characterized by off-odor and discoloration. Samples coated with CG and CGO-SNCs showed a slow microbial evolution throughout the entire experiment with no significant ($p > 0.05$) differences among samples. Here the microbial count for CG coated tomato is 1.6×10^5 CFU/mL and CGO-SNCs coated tomato shows only 1×10^5 CFU/mL for 10^{-3} dilution (Table 3).

However, the results were significantly different from the uncoated samples. This evidenced the microbial inhibitory effect of chitosan coating. Coating with CG and CGO-SNCs will provide a considerable level of antimicrobial activity which can prevent the growth of spoiling organisms and delays the spoilage of tomatoes. The antimicrobial effect of chitosan is thought to be related to the interaction between positively charged chitosan molecules and negatively charged microbial cell membranes[23].

According to Xu et al[42], a positive charge on the NH^{+3} group of glucosamine monomer at pH < 6.3 forms an electrostatic interaction with the negative charge of microbial cell membranes resulting in the leakage of intracellular components.

Conclusion

The use of nano-based chitosan solution enriched with orange oil will allow the retention of the physicochemical characteristics of tomatoes during its storage period. The coating of tomato using CGO-SNCs solution helped to prevent the over-ripening of fruit by maintaining the level of anthocyanin, lycopene, vitamin C, phenol content and flavonoid content. It also retained its antioxidant property during the entire storage period.

Furthermore, the coating methods helped to prevent bacterial growth to a larger extent and thereby enhanced the shelf life of tomatoes which has wide applications in the food industry.

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