Research Article

Chitosan-Cinnamon Oil Coating Maintains Quality and Extends Shelf Life of Ready-to-Use Pomegranate Arils under Low-Temperature Storage

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Different formulations of chitosan (1%, 2%, or 3%) with the incorporation of cinnamon oil (0.25% or 0.50%) were prepared for the preservation of pomegranate aril cv. Bhagwa. Six combinations of chitosan-cinnamon oil formulations along with one control (untreated) were applied to the freshly extracted arils using the dipping application method. All treatments were found to be effective in enhancing the shelf life, improving the postharvest characteristics, and reducing microbial populations on pomegranate arils during a 15-day storage period at 4 ± 1°C. The treated pomegranate arils exhibited excellent resistance to microbial decay, moisture loss, respiration rate, preservation of phenolics, flavonoids, and antioxidants activity, among other characteristics. Chitosan 2% + cinnamon oil 0.25% edible coating has a high potential to enhance the storage life and biochemical properties and reduce the microbial population of arils. This treatment recorded a higher total phenolic content (18%) and antioxidant activity (16%) than the control sample, respectively, at the end of storage. In addition, the treatment also helped to decrease the microbial activity by 45% compared to the control sample. The present investigation proposed an alternative method to prolong the shelf life of pomegranate arils during the 15 days of storage.

1. Introduction

India is the largest producer of pomegranate fruit in the world, followed by Iran. In a whole pomegranate fruit, the arils and seeds (edible parts) comprise 50%, while the peel consists of ~50% of the fruit [1, 2]. Over the recent years, there has been an extraordinary increase in consumer interest in pomegranate because of the high-quality attributes, unique flavor and taste, antioxidant properties of arils, and their health advantages [2–4]. Medicinal properties include antidiabetic, anticancerous, and antimicrobial activities. Additionally, it reduces obesity and maintains skin health [1]. It is widely consumed as fresh fruit and in processed form, such as jam, jelly, vinegar, wine, oil, and extract [4].

Nowadays, the demand for ready-to-eat pomegranate arils has increased due to the convenience of consuming and changing food consumption patterns. However, maintaining the nutritional and microbial quality of pomegranate arils is a major challenge since minimally processed arils easily lose quality characteristics such as texture and color, together with an increase in microbial spoilage [5, 6]. Only 10 d shelf life was observed in modified atmosphere (MA) packaged arils, and it was limited to only 7 d of the flavor and aroma [7].

Thus, new alternatives are required to reduce the microbial population on pomegranate arils and to delay quality loss. Edible coatings are emerging as one of the alternatives to preserve fresh and minimally processed commodities,
providing a partial barrier to moisture, oxygen, and carbon dioxide, improving mechanical handling properties, carrying additives, avoiding the loss of volatiles, and sometimes contributing to the production of aroma volatiles [8]. Edible coating is a thin and protecting layer, which acts as a carrier of natural ingredients for improving the postharvest quality of coated products by controlling oxidation, moisture loss, and deterioration effects [9]. Various plant- and animal-based edible biopolymers are used for the preparation of coating materials for applying to food materials. Chitin is the most abundant biopolymer in nature after cellulose. Edible coatings are widely used in the food processing sector as a thickener and preservative due to their functional properties, such as nontoxic nature, biodegradability, antimicrobial, and antioxidant characteristics [10]. The biopolymer-based edible coatings are one of the possible solutions to increase the shelf life of pomegranate arils and to maintain the nutritional, microbiological, and organoleptic properties.

Nevertheless, few studies have dealt with the preservation of pomegranate arils using various edible coatings, mainly chitosan coating. Özdemir and Gökmen [11] studied the effect of chitosan and ascorbic acid mixture as an edible coating on the shelf life extension of pomegranate arils. They reported that this coating helped to retain the visual quality of the arils during storage and inhibited bacterial and fungal growth on them. Recently, a number of materials having antimicrobial properties, such as essential oils (EO) from various sources, have been incorporated into the coatings and films used for fresh and fresh-cut fruits and vegetables [12, 13]. EOs from various plant sources serve as natural antimicrobials, and they are classified as generally recognized as safe (GRAS) [14, 15]. The possibility of an edible coating to carry EO is being studied because the oil as an antimicrobial agent can be released slowly from coating carriers to the food surface and the optimum oil concentrations can be maintained in the microenvironment. Furthermore, EO is included in the chitosan coating because it may substantially increase its antimicrobial properties for enhancing the shelf life of fruits [16]. The antimicrobial and antifungal properties of cinnamon oil (CO) have drawn great attention from many researchers [17, 18]. CO had antifungal properties of cinnamon oil (CO) have drawn great attention from many researchers [17, 18]. CO had been demonstrated high fungicidal activity against Fusarium moniliforme [19]. CO (ranging between 25 and 500 ppm) was tested for antifungal activity against Colletotrichum coccodes, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer, and Aspergillus niger in vitro. Oil enrichment resulted in a reduction of subsequent colony development for the examined pathogens [17].

Therefore, the main objective of this investigation was to evaluate the effect of chitosan-cinnamon oil coating on the postharvest physiological attributes, preservation quality, and shelf life of the pomegranate arils during refrigerated storage conditions (4 ± 1°C).

2. Materials and Methods

2.1. Materials. Pomegranate (Punica granatum L. cv. “Bhagwa”) fruits were harvested at commercial maturity (TSS 17.0 ± 0.50%, pH 4.2 ± 0.17, % citric acid 1.85 ± 0.15) from a farmer’s orchard, New Delhi, India. Fresh, regular-shaped, uniform-sized, healthy fruits (without defects) were phytosanitized by washing in a commercial pomegranate fungicide (Teacher™ solution at 600 ppm) for 3 min and allowing the fruit to dry at room temperature. The fruits were immediately stored in a cold room at 5 ± 1°C before the experiment.

Black polypropylene terephthalate (PET) punnet sample containers (14.5 × 19.0 × 4 cm) and low-density polyethylene (LDPE) film were purchased from Friends Enterprises (New Delhi, India). The thickness of the PET punnets and LDPE films was 1.52 ± 0.03 mm and 49.915 ± 0.05 μm, respectively. Water vapor, O2, and CO2 transmission rates of PET punnets were 27.2 g m−2 day−1, 60 ± 5 cm−2, and 25 ± 3 cm−2, respectively, while the water vapor, O2, and CO2 transmission rates of LDPE film were 18.25 g m−2 day−1, 31410 ± 1050 cm−3 m−2, and 8505 ± 510 cm−3 m−2, respectively.

2.2. Chemicals and Solvents. Plate count agar (PCA), solvents (HPLC grade methanol), 2,2-diphenyl-picrylhydrazyl (DPPH*), cyanidin 3-glucoside, Folín–Ciocalteu (FC) reagent, metaphosphoric acid, 2,6-dichlorophenolindophenol (dye), and polyvinylpolypyrrolidone (PVP) were purchased from Sigma-Aldrich (New Delhi, India). Low molecular chitosan (40 kDa, >75% deacetylation), CO (extracted from bark, 99.9% purity), Tween-80, and other chemicals were purchased from Hi Media Ltd. (Mumbai, India).

2.3. Sample Preparation. Pomegranate fruits, plastic containers, knives, and utensils were sterilized with 70% ethanol, followed by sodium hypochlorite washing. After washing, the fruit rind was carefully cut at the equatorial zone with a sharp sterilized knife, and arils were manually extracted in a sterilized laminar flow (Maxisafe-2030i, Thermo Scientific, Mumbai, India) to reduce the contamination. Arils were collected in plastic crates and washed with sterile water for 5 min and drained excess water from arils with sterilized paper. The samples were prepared at room temperature (20 ± 2°C).

2.4. Formulation and Preparation of Coating Materials. Biopolymer chitosan (1, 2, or 3%)-based edible coatings were prepared by adding CO at 0.25% and 0.5% concentrations with 0.5% Tween-80 (v/v). The chitosan polymer was mixed at 1, 2, or 3% concentrations in a 0.5% aqueous solution of citric acid (w/v). The prepared solutions were stirred for 1 h at room temperature (25 ± 1°C) in a magnetic stirrer (IKA, Germany; RH Basic 1). Furthermore, the final solutions were homogenized at 10,000×g for 5 min at room temperature before the coating of arils (Table 1).

2.5. Application of Coating and Storage. The separated arils (17.5 kg: 2.5 kg for each group) were randomly divided into seven groups and dipped into seven different coating solutions of 500 mL for 5 min at 20°C (Table 1). Furthermore, the treated arils were strained and kept for drying at room
temperature for 3 h and then packed in plastic punnets (plastic containers). Punnets were kept in cold storage (4 ± 1°C, 90–95% RH) for further investigations.

2.6. Physiological and Physical Attributes

2.6.1. Respiration Rate. The respiration rate of treated arils was determined using a closed system method [20] using a headspace analyzer (PBI Dansensor CheckMate II Headspace Gas Analyzer, Denmark). Arils were weighed using a weighing balance (Citizen, Mumbai, India). Arils were packed in air-tight glass jars and sealed. The volume of the jar was measured. A hole was made on the lid of the jar, and it was packed with rubber septa. After 2 h of packing, the probe of the analyzer was inserted into the jar through the septa. The CO₂ concentration was recorded in percentage and calculated using the following formula:

\[ \text{CO}_2 = \frac{\text{Net CO}_2}{1000} \times \text{Headspace} \times \frac{1000}{m} \times \frac{60}{t}, \]

where Net CO₂ = CO₂ (fruit) – CO₂ (ambient), Headspace is in mL, m denotes the sample mass, and t denotes the time of incubation.

2.6.2. Weight Loss. Weight losses of the samples were determined using a digital balance (BSA224S-CW, Sartorius, Germany). The samples were then kept in a plastic tray for measuring weight loss every 3 d. Weight loss was expressed as a percentage (%) and calculated using the following formula:

\[ \text{WL} (%) = \frac{W_i - W_f}{W_i} \times 100, \]

where \( W_i \) is the initial weight and \( W_f \) is the final weight.

2.6.3. Color. Color coordinates of the samples were determined using a hand-held chroma meter (CR-400, Konica Minolta, Japan), which provided CIE \( L^* \), \( a^* \), and \( b^* \) measurements. \( L^* \) defines the lightness, and \( a^* \) and \( b^* \) define red-greenness and blue-yellowness, respectively. A calibrated apparatus with a white tile background (illuminants C: \( Y = 93.6, x = 0.3133, \) \( y = 0.3195 \)) was used for measurement. Three measured color parameters (\( L^* \), \( a^* \), and \( b^* \)) were converted into chroma (\( C^* \)) and hue angle (\( h^\circ \)). The chroma and hue angles were calculated using the following formula:

\[ \text{C}^* = \left( (a^*)^2 + (b^*)^2 \right)^{0.5}, \]

\[ h^\circ = \tan^{-1} \left( \frac{b^*}{a^*} \right). \]

2.7. Chemical and Enzymatic Attributes

2.7.1. Titratable Acidity, pH, and Total Soluble Solids. The titratable acidity (TA) of arils was determined according to the method of Meighani et al. [21]. Aril juice (2 mL) was homogenized (10 mL water) and titrated with 0.1 N NaOH. The results were expressed as the percentage of citric acid using the following formula:

\[ \text{TA} \text{ (%)} = \frac{\text{titre value} \times \text{normality of NaOH} \times \text{eq. weight of acid} \times 100}{\text{volume of sample taken} \times \text{aliquot volume} \times 1000}. \]

The pH of arils was determined using a hand-held pH meter (HI, 2221, Hanna, Rhode Island, US). TSS of the juice obtained from the arils (5 g from each group) was determined using a digital refractometer (Atago, Tokyo, Japan), and the results were expressed as %.

2.7.2. Enzyme Assays. Arils (5 g) were homogenized with 10 mL of extraction buffer (100 mM L⁻¹ potassium phosphate buffer, pH 7.0, 0.5 mM L⁻¹ EDTA, 60 g L⁻¹ PVP) for 20 min to determine the enzymatic activity. The mixtures were centrifuged at 15,000 x g, and the supernatants were used to determine the enzyme activity in terms of polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) assays [22].

2.7.3. Polyphenol Oxidase (PPO). The activity of PPO (EC 1.14.18.1) was determined by the method of González et al. [23]. Three mL of the reaction combination consisting of 2.5 mL of potassium phosphate buffer (pH 6.0), 0.3 mL of 0.5 M pyrocatechol, and 0.2 mL of crude enzyme was tested...
for the activity. The blank consisted of a phosphate buffer of 3 mL potassium (pH 6.0), and the results of PPO were expressed as U g$^{-1}$.

2.7.4. Phenylalanine Ammonia Lyase (PAL). PAL (EC 4.3.1.25) activity was determined by Meighani et al. [21] with some modifications. Enzyme extract (500 μL) was mixed with 2 mL of 50 mM borate buffer (pH 8.8) and 500 μL of 20 mM L-phenylalanine at 37°C for 60 min. HCl (6 N) was used in 100 μL volume to stop the reaction. The production of cinnamate was measured by absorbance at 290 nm. The specific activity of the enzyme was described as the nmol cinnamic acid h$^{-1}$ g$^{-1}$.

2.8. Phytochemical Content

2.8.1. Total Phenolic Content (TPC). The TPC was measured according to the FC method [20] with minor modifications. Aril extract (1 mL) dissolved with 70 mL of distilled water and 5 mL of FC reagent (10-fold dilution) were added and kept for 2 min. Thereafter, 15 mL of sodium carbonate (20%) was added, and the volume was made up to 100 mL with distilled water. After incubation (2 h), the absorbance was recorded at 765 nm using a spectrophotometer (Sican, 2301, Incarp Instrument Ltd., India). Gallic acid was used as a standard, and the results were shown as gallic acid equivalent (g GAE kg$^{-1}$).

2.8.2. Total Flavonoid Content (TFC). The TFC was measured using the aluminum chloride spectrophotometric method with some modifications [21]. The extraction was done from a 5 g sample using 50 mL methanol. Sample extract (1 mL) was added to 4 mL of distilled water and 0.3 mL of 5% NaNO$_2$. After 5 min, 0.3 mL of 10% AlCl$_3$ was added to the solution. Furthermore, 2 mL of 1 M NaOH was added, and the mixture volume was made up to 10 mL with distilled water. The absorbance was measured at 430 nm using a UV-Vis spectrophotometer. Quercetin was used as a standard, and the results were expressed as QE mg 100 g$^{-1}$.

2.8.3. Total Anthocyanin Content (TAC). TAC was determined by the pH differential method of Boussaa et al. [24] with two buffer solutions. Pomegranate juice (1 mL) was diluted in 9 mL of two different buffers: potassium chloride

Figure 1: Effect of chitosan coating and cinnamon essential oil on the respiration rate of pomegranate arils during storage at 4 ± 1°C. Vertical bars represent the mean ± SD of 3 replicates. Error bars with different letters on the same storage period show a significant difference (P ≤ 0.05). T0 = control; T1 = 1% chitosan + 0.25% oil; T2 = 1% chitosan + 0.5% oil; T3 = 2% chitosan + 0.25% oil; T4 = 2% chitosan + 0.5% oil; T5 = 3% chitosan + 0.25% oil; T6 = 3% chitosan + 0.5% oil.
**Figure 2:** Effect of chitosan coating and cinnamon essential oil on weight loss of pomegranate arils during storage at 4 ± 1°C. Vertical bars represent the mean ± SD of 3 replicates. Error bars with different letters on the same storage period show a significant difference (P ≤ 0.05). T0 = control; T1 = 1% chitosan + 0.25% oil; T2 = 1% chitosan + 0.5% oil; T3 = 2% chitosan + 0.25% oil; T4 = 2% chitosan + 0.5% oil; T5 = 3% chitosan + 0.25% oil; T6 = 3% chitosan + 0.5% oil.

**Table 2:** Effect of chitosan and cinnamon oil coatings on chroma (C*) and hue angle (h°) of pomegranate arils during storage.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Chroma (C</em>)</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>22.12 ± 1.33d</td>
<td>19.81 ± 1.09a</td>
<td>18.18 ± 0.49b</td>
<td>17.45 ± 1.45a</td>
<td>13.54 ± 1.39a</td>
<td>10.32 ± 1.46a</td>
</tr>
<tr>
<td>T1</td>
<td>20.76 ± 1.81b</td>
<td>20.75 ± 0.65b</td>
<td>20.44 ± 0.77d</td>
<td>19.61 ± 0.77c</td>
<td>18.72 ± 0.62c</td>
<td>17.38 ± 0.56d</td>
</tr>
<tr>
<td>T2</td>
<td>19.45 ± 3.12a</td>
<td>19.81 ± 0.82a</td>
<td>19.71 ± 0.98c</td>
<td>17.65 ± 0.51a</td>
<td>17.61 ± 0.58b</td>
<td>17.03 ± 0.80d</td>
</tr>
<tr>
<td>T3</td>
<td>19.45 ± 3.12a</td>
<td>22.12 ± 0.78d</td>
<td>21.13 ± 0.73d</td>
<td>20.69 ± 1.13d</td>
<td>19.74 ± 0.56d</td>
<td>18.09 ± 0.71e</td>
</tr>
<tr>
<td>T4</td>
<td>21.26 ± 1.27c</td>
<td>21.46 ± 1.25c</td>
<td>20.96 ± 0.90d</td>
<td>20.58 ± 1.19d</td>
<td>19.27 ± 0.71d</td>
<td>17.29 ± 0.86d</td>
</tr>
<tr>
<td>T5</td>
<td>19.43 ± 0.30a</td>
<td>19.79 ± 0.69a</td>
<td>16.85 ± 4.82a</td>
<td>18.21 ± 1.06b</td>
<td>17.21 ± 0.83b</td>
<td>15.39 ± 1.92c</td>
</tr>
<tr>
<td>T6</td>
<td>19.53 ± 0.43a</td>
<td>19.79 ± 0.83a</td>
<td>19.59 ± 3.16c</td>
<td>18.18 ± 1.48b</td>
<td>17.03 ± 0.51b</td>
<td>13.7 ± 0.77b</td>
</tr>
<tr>
<td><strong>Hue angle (h°)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>0.31 ± 0.01c</td>
<td>0.40 ± 0.01b</td>
<td>0.30 ± 0.04a</td>
<td>0.25 ± 0.03b</td>
<td>0.21 ± 0.03b</td>
<td>0.16 ± 0.04b</td>
</tr>
<tr>
<td>T1</td>
<td>0.30 ± 0.01a</td>
<td>0.36 ± 0.00c</td>
<td>0.33 ± 0.02d</td>
<td>0.29 ± 0.02d</td>
<td>0.25 ± 0.02c</td>
<td>0.17 ± 0.05c</td>
</tr>
<tr>
<td>T2</td>
<td>0.27 ± 0.06b</td>
<td>0.38 ± 0.01f</td>
<td>0.29 ± 0.02c</td>
<td>0.27 ± 0.00c</td>
<td>0.21 ± 0.01b</td>
<td>0.26 ± 0.04e</td>
</tr>
<tr>
<td>T3</td>
<td>0.30 ± 0.01a</td>
<td>0.34 ± 0.06d</td>
<td>0.37 ± 0.13e</td>
<td>0.34 ± 0.12f</td>
<td>0.31 ± 0.12e</td>
<td>0.29 ± 0.08f</td>
</tr>
<tr>
<td>T4</td>
<td>0.31 ± 0.01c</td>
<td>0.33 ± 0.06d</td>
<td>0.33 ± 0.09d</td>
<td>0.31 ± 0.06e</td>
<td>0.29 ± 0.03d</td>
<td>0.25 ± 0.01d</td>
</tr>
<tr>
<td>T5</td>
<td>0.30 ± 0.01a</td>
<td>0.32 ± 0.02c</td>
<td>0.26 ± 0.07b</td>
<td>0.25 ± 0.03b</td>
<td>0.21 ± 0.06b</td>
<td>0.16 ± 0.12b</td>
</tr>
<tr>
<td>T6</td>
<td>0.29 ± 0.02b</td>
<td>0.03 ± 0.97a</td>
<td>0.25 ± 0.07b</td>
<td>0.23 ± 0.01a</td>
<td>0.20 ± 0.02a</td>
<td>0.15 ± 0.06a</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SD (n = 3) (P ≤ 0.05). a, b, c, d, and e indicate significant differences in treatments compared to control with respect to an interval of 3 days (storage days 0, 3, 6, 9, 12, and 15 days, resp.). T0 = control, T1 = chitosan 1% + cinnamon oil 0.25%, T2 = chitosan 1% + cinnamon oil 0.50%, T3 = chitosan 2% + cinnamon oil 0.25%, T4 = chitosan 2% + cinnamon oil 0.50%, T5 = chitosan 3% + cinnamon oil 0.25%, and T6 = chitosan 3% + cinnamon oil 0.50%.
and sodium acetate (0.4M, pH 4.5). The mean results of anthocyanin were expressed as mg 100g⁻¹.

The absorbance (A) of the two dilutions was recorded at 510 and 700nm, respectively, and calculated by the following formula:

\[
TAC = \frac{A \times MW \times 1000}{\varepsilon \times 10},
\]

where \( A = [(A_{510\text{nm}} - A_{700\text{nm}}) \text{ at pH 1.0} - (A_{510\text{nm}} - A_{700\text{nm}}) \text{ at pH 4.5}] \), dilution factor (DF) of 10, an extinction coefficient (\( \varepsilon \)) of 26,900L mol⁻¹ cm⁻¹, and a molecular weight (MW) of 449.2g mol⁻¹.

2.9. Antioxidant Content. Determination of free radical scavenging activity (RSA) (through DPPH⁺) was determined spectrophotometrically according to the procedure described by Maurya et al. [25] with some modifications. Fresh pomegranate juice (0.1mL) was mixed with 0.9mL of 100mM Tris-HCl buffer (pH 7.4), to which 1mL of DPPH (500μM in ethanol) was added. After incubation (30 min), the absorbance was measured at 517nm using a UV-Vis spectrophotometer. The reaction mixture without DPPH was used for background correction. The RSA (antioxidant activity) was calculated using the following equation:

\[
\text{antioxidant activity (\%)} = 1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100.
\]

2.10. Antimicrobial Activity. For shelf life estimation, a microbial analysis (total plate count) was performed for five random samples with 6, 12, or 15d of storage. A sample (30g) from each group was blended with 270mL of sterile peptone-buffered water for 1 min in a sterile stomacher bag using a masticator. Samples were diluted decimally, and 100μL was spread on plate count agar (PCA) plates for the enumeration of viable microorganisms, which were incubated at 20°C for 24h [26]. Colony count was reported as Log₁₀ colony-forming units (CFU) mL⁻¹ sample.

2.11. Sensory Evaluation. The sensory characteristics of arils were evaluated by a panel of staff, students, and faculty of the National Institute of Food Technology, Entrepreneurship...
and Management (NIFTEM), Kundli, India, between the age of 17 to 55 (female: male = 1:1, n = 60). A 9-point hedonic scale (1 = dislike extremely; 9 = like extremely; 5 = acceptable limit) was used to record the score of liking and disliking of important parameters, such as color, flavor, body texture, and overall acceptability. Sensory evaluation was done from 0 to 15 d at the interval of 3 d. L/ he analysis was performed in a cabin with illuminating light, and potable water was provided for palate cleansing [27].

2.1.2. Statistical Analysis. All experiments were analyzed in triplicate, and the results are presented in mean ± SD on a fresh weight basis (FW). The data were analyzed through one-way analysis of variance (ANOVA), and the differences between the mean values were analyzed by Post Hoc: Duncan’s multiple range test at \( P \leq 0.05 \) significance level. The data analysis was done using IBM SPSS (20.0 version) software (Chicago, USA).

3. Results and Discussion

3.1. Physiological and Physical Attributes

3.1.1. Respiration Rate. The higher respiration rate of arils depends on the temperature and storage time. The investigation revealed that initially, during the storage, treated pomegranate arils had a lower respiration rate, which increased with the storage period (Figure 1). A significant difference was observed in respiration rate between treated and control pomegranate arils during the storage period. A marked difference was recorded in the respiration rate at the end of storage in all treatments. At 15 d of storage, the highest respiration rate (5.62 mg CO\(_2\) kg\(^{-1}\) h\(^{-1}\)) was recorded in the control and lowest (3.42 mg CO\(_2\) kg\(^{-1}\) h\(^{-1}\)) with chitosan 2%+CO 0.5% treatment (T4). This happened probably due to the reducing gas interchange and consequently low oxygen availability to the fruit tissues for respiration [28]. Moreover, the results showed that the use of
CO in the coatings progressively reduces the respiration rate. Two factors, namely, the absence of physical barrier (coating) and the occurrence of chilling injury, mainly contributed to the comparatively higher respiration rate in the control group [9]. A study reported a reduced respiration rate in coated fruits and vegetables during storage due to the slow metabolism [8, 21]. The results are consistent with previous studies investigating the chitosan coating effect on pomegranate arils during the storage period [22, 29]. Yousuf and Srivastav [15] observed lower CO₂ production for arils coated with flaxseed gum (0.3 and 0.6%) enriched with lemongrass oil (0–800 ppm) when compared to uncoated arils. This was due to the inhibitory effects of flaxseed gum on the respiration rate for all coated arils. Similarly, a lower respiration rate in “Acco” pomegranate arils coated with methylcellulose and gum arabic enriched with thyme oil was observed by Kawhena et al. [10].

3.1.2. Weight Loss. Weight loss of the arils was measured at an interval of 3 d up to 15 d of storage (Figure 2). Weight loss mainly occurs due to water loss caused by transpiration in fresh fruits and vegetables [7]. Therefore, the coating was applied to prevent water loss and to improve the postharvest physiological conditions of fruits. The weight loss was less in the treated arils compared to the control, which shows the effectiveness of the coating material in maintaining the weight loss of arils. The inhibition of water vaporization and decreased metabolic process with lower respiration rate were the main reasons behind this [30]. At the end of cold storage (15 d), the control arils lost 11.70% weight; however, it was found that the less amount of cinnamon oil (0.25%) with 2% chitosan was found most effective in preventing moisture loss during storage. Results are consistent with the previous study of Zahran et al. [29] who reported that the chitosan coating has a significant role in the retention of weight loss in
pomegranate arils during storage. Likewise, Özdemir and Gökmen [11] coated pomegranate arils with chitosan (1 or 2%) and ascorbic acid (1%) and observed a controlled weight loss during 28 d of storage, which was under the commercial limit. Sason and Nussinovitch [31] also reported a lower weight loss (9%) in alginate-coated pomegranate arils compared to the control arils (11%) on 30 d of storage at 4°C.

### 3.1.3. Color

Color attributes of fresh produce influence consumer’s choice and preferences. Cultivar differences have also played a role in the way color attributes change over time [6]. This is consistent with the observation that, in this current study, cv. “Bhagwa” had comparably lower color intensity reflected by chroma (C∗) of arils (Table 2). Storage duration was the main factor for the changes observed in the aril chroma (C∗) and hue angle. Furthermore, the coating had effects on the hue angle and chroma due to the controlled activity of PPO enzymes [32]. Moreover, the interaction between chitosan treatment and the storage duration was also significant for hue angle. These findings are consistent with other reports on the color changes of minimally processed pomegranate aril [6, 11, 20]. Similarly, Fawole and Opara [33] have reported that chitosan treatment delays the color changes and controls the browning of pomegranate arils.

Table 2 illustrates the change of hue angle (h°) of treated arils during the storage period. Hue angle can be an indication of the appearance of aril browning. Regardless of preharvest treatment, the values of skin color (hue angle) of pomegranate arils decreased significantly in correlation with time progress. Control samples recorded the highest value of hue angle up to 3 d of storage. Thereafter, T3 treatment (chitosan 2% + CO 0.25%) showed the highest hue angle values (0.37 ± 0.13–0.29 ± 0.08), followed by T4 treatment (chitosan 2% + cinnamon oil 0.5%) up to 15 d of storage. Overall, the results showed that there was no significant effect of coating observed on the hue angle of the arils during the cold storage. Similar results were reported by Vargas et al. [34] in strawberry fruits during storage treated with a chitosan-oleic acid edible coating.

### 3.2. Chemical and Enzymatic Attributes

#### 3.2.1. Titratable Acidity, pH, and Total Soluble Solids

TA gradually increased with the storage period (Figure 3). The TA ranged from 0.303 to 0.481 for all samples on day 3 of storage. Treatment T2 (1% chitosan + 0.50% CO) was more effective than other treatments. Probably, the breakdown and fermentation of sugar contents in pomegranate arils increased the TA during storage [35]. The treated samples had a lower increase in TA, which is consistent with the
previous studies [6, 28]. Recently, Hasheminejad and Khodaiyan [36] found the highest level (0.83%) of TA in chitosan nanoparticles and clove EO- (ChNP-CEO-) coated arils compared to control (0.62%) when stored at 5°C for 55 d.

The coated arils showed a smaller decrease in pH value (3.5) in comparison to the control samples (Figure 4). However, there was no significant ($P < 0.05$) difference detected between control and treated arils. The pH ranged from 3.71 to 3.85 and on day 15 from 3.5 to 3.64 for all treated samples on day 3. A decrease in pH was noticed during the storage period, which can be attributed to the formation of acid due to the breakdown and fermentation of sugars in pomegranate arils. The highest pH value was found for T6 treatment (3.64), followed by T4 treatment (3.63). The results indicated that the coating has the ability to slow down the pH change in arils for a long storage period, while chitosan:ascorbic acid (1:1 w/w) coating gradually increased the pH of arils from 3.93 to 4.05 during 28 d of cold storage (5°C) [11].

The TSS values for all samples showed an increase, indicating the hydrolysis of starch into sugars which is desirable in case of pomegranate juice. TSS of control samples showed a higher increase compared to coated, which might be due to the lower rate of respiration (Figure 5). T4 treatment had the lowest TSS increase, showing the best organoleptic property among all samples. The TSS was gradually increased in the control arils as compared to chitosan-treated arils. It occurred due to the utilization of reducing sugar and other organic metabolites. Das et al. [37] reported that the chitosan coating reduced the organic metabolites and respiration rate, consequently slower hydration of sugar molecules of fruits and vegetables during storage. Additionally, Salama et al. [38] reported similar trends in EO-coated pomegranate arils during storage. Hasheminejad and Khodaiyan [36] also reported the highest TSS in the ChNP-CEO-treated aril (17 Brix) when stored at 5°C for 55 d.

### 3.2.2. Polyphenol Oxidase (PPO)

A decrease in phenol concentration is correlated with increased PPO activity and browning, which was caused by the enzymatic oxidation of phenols in arils [39]. PPO catalyzes the hydroxylation of monophenol to diphenols and oxidation of diphenol to diquinone accompanied by nonenzymatic melanin production. The results showed a positive correlation between TPC and both activities of PPO enzymes. The PPO activity in
the control arils (T0) showed an increasing trend, which was up to 43.11 U g\(^{-1}\), at the end of storage. Chitosan-cinnamon oil coating resulted in the lowest PPO activity in T3 samples (25.85 U g\(^{-1}\)) after 15 d of storage (Figure 6). Jiang [40] proposed that PPO initially catalyzes the degradation of phenols, anthocyanidins, and/or their degraded derivatives into quinones during the pericarp browning of litchi fruits, resulting in browning and declining anthocyanin content. The browning event in the aril led many customers to doubt their purchase. However, the chitosan coating may reduce anthocyanin degradation during the storage of arils and inhibit PPO activity. The results are in accordance with the results of Ghasemnezhad et al. [22], who inhibited browning and polyphenol oxidation by chitosan coating on arils during storage. Zarbakhsh et al. [41] reported a decrease in the PPO activity of pomegranate (cv. “Jahrom”) arils treated with citric acid (1.63 ± 0.0.39 U mg\(^{-1}\)) or ascorbic acid (1.15 ± 0.09 U mg\(^{-1}\)) compared to water (5.80 ± 0.65 U mg\(^{-1}\)) under storage at 5–7°C for 15 d.

3.2.3. Phenylalanine Ammonia Lyase. The activity of PAL increased in the control compared to the treated arils (Figure 7). PAL catalyzes the conversion of phenylalanine to trans-cinnamic acid as a key enzyme in the phenylpropanoid pathway [41]. Besides control, the highest PAL activity was observed in the arils treated with T6, followed by T5 treatment. The concentration and ratio of CO in the coating treatments were the reason behind the higher PAL activity. Furthermore, microbial count in treated arils was reported to be low, and in response to the development and ripening, PAL has been reported to be transcriptionally induced. The lowest production of PAL enzyme was recorded in the arils treated with T3 (23.28 h\(^{-1}\) g\(^{-1}\)) and T4 (23.46 h\(^{-1}\) g\(^{-1}\)) treatments at the end of storage. The control arils showed a higher enzyme production (PAL) compared to treated arils. Dávila-Aviña et al. [42] reported that the edible coating has an effect on the enzymatic activity and metabolic pathways in tomato.

3.3. Phytochemical Content

3.3.1. Total Phenolic Content (TPC). Pomegranate juice is a rich source of phenolic compounds, having synergistic and/or additive effects on pharmacological activities [43]. Results showed a reduction attributed to PPO activity on phenols leading to their oxidation. There was 69.53% reduction in the control samples compared to the TPC of treated arils (52.44% to 66.9%) (Figure 8). The chitosan and CO coating suppressed the reduction of phenolic content. The decreased
TPC leads to browning; thus, the browning rate can be reduced using chitosan and CO as a coating material. The least reduction in TPC level was found in treatment T3. Seow et al. [44] reported an increase in permeability of the cell membrane due to strong interactions of an EO. The EO can also control the enzymatic activity and oxidation of phenolic compounds. Kanatt et al. [45] maintained TPC using chitosan coating by minimizing the oxidation and browning of the produces. The results also agreed with the previous study of Xing et al. [18], who controlled the phenolic activity of jujube fruits using chitosan-CO coating during storage. Similarly, Abdel Fattah et al. [35] revealed that the application of chitosan coating delayed the decrease in phenolics content in pomegranate arils during cold storage and also showed that chitosan had slight or no significant \((P \leq 0.05)\) changes in chemical, microbiological, and sensory quality. Zarbakhsh et al. [41] also found the highest \((18.50 \pm 0.27 \text{ mg GAE g}^{-1})\) TPC in ascorbic acid-treated arils when stored for 15 d at 5–7°C.

3.3.2. Total Flavonoid Content (TFC). The change in TFC was found to be similar to TPC. The TFC was reduced for both coated and uncoated samples (Figure 9). The TFC was decreased in the range of 2.85–3.39 (3 d) to 1.52–1.97 (15 d). It showed that the coating material slowed down the reduction of TFC. The least TFC reduction was obtained in T3 treatment. A significant difference \((P < 0.05)\) was observed between all coating treatments. The flavonoid content is responsible for the antioxidant activity of the fruit; thus, evident treatment T3 had more antioxidant activity. The results are supported by the previous study on guava fruit [46]. Thus, the chitosan-CO edible coating has the potential to maintain the carotenoid compounds of arils during storage. Xing et al. [18] controlled the flavonoid loss using chitosan coating on fruits and vegetables during storage.

3.3.3. Total Anthocyanin Content (TAC). The samples were evaluated for TAC in an interval of 3 d up to 15 d. A significant difference \((P < 0.05)\) was found in the treated samples. Anthocyanin is not stable and susceptible to oxidation, followed by browning in arils during storage. The results showed a reduction in anthocyanin content from 326.56–387.6 mg 100g\(^{-1}\) to 200.89–269.75 mg 100g\(^{-1}\) (Figure 10). However, the coating treatments maintained the TAC for a longer period than the control. The least reduction was found in treatment T4 (269.75 mg 100g\(^{-1}\)), followed by...
T3 (261.87 mg 100g$^{-1}$) due to the increased CO concentration. TAC reduced slowly up to 9 d; thereafter, it decreased rapidly up to 15 d of storage, indicating increased activity of the oxidative enzyme (Figure 10). The coating acted as a barrier against lipid oxidation and reduced the gas metabolism [41]. The results are consistent with the studies of Fattah et al. [35] and Özdemir and Gökmen [11], who reported delayed degradation of TAC in chitosan and chitosan-ascorbic acid-coated arils during storage. Similarly, Zarbakhsh et al. [41] observed a gradually reduced TAC with the highest anthocyanin content (0.04 ± 0.005 mg 100g$^{-1}$) in ascorbic acid-treated arils at the end of the experiment. Further, Varasteh et al. [28] found that “Rabbabe Neyriz” pomegranates treated with 1% and 2% chitosan had greater anthocyanin content in pomegranate arils, indicating that di-glucoside anthocyanins are more immutable than monoglucoside anthocyanins.

3.4. Antioxidant Content. The reduction in antioxidant activity was found from 81.37 (0 d) to 42.87–57.54 (15 d). There was a significant difference ($P < 0.05$) between different treatments (Figure 11). The coating effectively slowed down the reduction of antioxidant activity. The reduction in antioxidants was least in T3-coated arils together with the highest phenolic content. Additionally, the coating treatments with 0.25% (T1, T3, or T5) CO have a better antioxidant value after 15 d storage than the coating treatments with 0.50% (T2, T4, or T6). The applied coating may prevent phenol oxidation in arils during the storage period. It also showed that the chitosan coating helped to maintain the antioxidant property of the arils due to the addition of CO [47]. The results are in accordance with the study of Fattah et al. [35], who maintained antioxidant activity in chitosan-treated arils during storage. Moreover, Saba and Amini [48] observed a significant increase in the antioxidant activity of nano-ZnO-added carboxymethyl cellulose- (ZnO-CMC-) treated arils (75%) than control (65%) at 4°C for 12 d. They also found that the coating material kept intact the phenols and flavonoids of the arils. Recently, Zarbakhsh et al. [41] reported a higher increase in the antioxidant activity of pomegranate (cv. “Jahrom”) arils treated with citric acid (72.55 ± 3.53%), ascorbic acid (79.69 ± 7.50%), and chitosan (60.58 ± 5.51%) than water (62.08 ± 11.38%) at 5–7°C storage for 15 d.

![Figure 10: Effect of chitosan coating and cinnamon essential oil on total anthocyanin content (3 d) of pomegranate arils during storage at 4 ± 1°C. Vertical bars represent the mean ± SD of 3 replicates. Error bars with different letters on the same storage period show a significant difference ($P \leq 0.05$). T0 = control; T1 = 1% chitosan + 0.25% oil; T2 = 1% chitosan + 0.5% oil; T3 = 2% chitosan + 0.25% oil; T4 = 2% chitosan + 0.5% oil; T5 = 3% chitosan + 0.25% oil; T6 = 3% chitosan + 0.5% oil.](image-url)
3.5. Microbial Stability of Arils. Initially, all treatments exhibited similar total aerobic plate counts (TAPC). This indicates the maintenance of good hygienic conditions during manual extraction and coating of arils. Furthermore, the microbial load was comparatively increased on 12d of storage. On 18d, the lowest TAPC growth was recorded in T4 treated arils (4.34log CFU mL\(^{-1}\)), followed by T3 treated arils (4.72log CFU mL\(^{-1}\)), while the highest growth of microbes was recorded in the control (T0), that is, 9.68log CFU mL\(^{-1}\). Comparatively, treatments T3 and T4 significantly inhibited the microbial growth in arils during the storage period (Figure 12). The polyphenols of coating material are the reasons behind the inhibition of microbes. Several studies confirmed the significant effect of chitosan and EO treatment on food microbes because of their phenolic compounds [22, 49]. The results are in line with the studies done by Fattah et al. [35] in chitosan-coated pomegranate arils and Özdemin and Gökmen [11] in chitosan-ascorbic acid-coated pomegranate arils. Saba and Amini [48] also reported the least bacterial load (2.2 log CFU g\(^{-1}\)) in ZnO-CMC treated pomegranate arils than the control (2.7 log CFU g\(^{-1}\)) at 4°C storage for 12 d. The inclusion of cinnamon essential oil in the coating showed greater antimicrobial activity [44].

3.6. Sensory Evaluation. At 0 d, the control arils were under acceptable limits in terms of freshness, color, texture, taste, and general acceptance (Table 3). On 0 d onwards, the highest scores were given to the arils of T3 (chitosan 2%+CO 0.25%). Furthermore, as a result of coating, the panelists did not perceive any off-flavor in arils. As verified by the surface, the chitosan coating maintained the visual quality of the arils during storage. The panelists gave preferences to chitosan-treated arils compared to the control, and the control samples were restricted to 12 d while treating arils up to 15 d. Similar findings were reported by Kumkum et al. [50] as a result of Aloe vera gel and CO-based coating on the sensory characteristics of pomegranate arils during storage. Similarly, Özdemin and Gökmen [11] observed that the chitosan-ascorbic acid (1:1 w/w) coating kept the arils comparatively fresh than the control in terms of color, freshness, taste, aroma, texture, and overall acceptability up to 14 d of cold storage (5°C), which supported...
Figure 12: Effect of chitosan coating and cinnamon essential oil on total plate count (log CFU mL\(^{-1}\)) of pomegranate arils during storage at 4 ± 1°C. Vertical bars represent the mean ± SD of 3 replicates. Error bars with different small letters on the same storage period show a significant difference (\(P \leq 0.05\)). T0 = control; T1 = 1% chitosan + 0.25% oil; T2 = 1% chitosan + 0.5% oil; T3 = 2% chitosan + 0.25% oil; T4 = 2% chitosan + 0.5% oil; T5 = 3% chitosan + 0.25% oil; T6 = 3% chitosan + 0.5% oil.

Table 3: Effect of chitosan and cinnamon essential oil coatings on sensory evaluation of pomegranate arils during storage.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Treatments</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
<th>Day 15</th>
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<tbody>
<tr>
<td>Freshness</td>
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<td>7.2 ± 0.63b</td>
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<tr>
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<td>8.0 ± 0.66a</td>
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<td>5.7 ± 1.18ab</td>
<td>5.0 ± 0.66c</td>
</tr>
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</table>

| Color           | T0         | 8.8 ± 0.42a | 8.1 ± 0.73ab | 7.6 ± 0.96a  | 6.1 ± 1.17ab | 5.2 ± 0.60bc | 4.4 ± 0.48d  |
|                 | T1         | 8.5 ± 0.52a | 7.9 ± 0.73ab | 7.1 ± 0.73a  | 6.5 ± 1.17ab | 5.9 ± 0.56ab | 5.8 ± 0.63ab |
|                 | T2         | 8.5 ± 0.52a | 7.6 ± 0.51b  | 7 ± 0.81a    | 6.5 ± 0.52ab | 5.8 ± 0.63ab | 5.4 ± 0.51bc |

|                | T3         | 8.6 ± 0.51a | 8.4 ± 0.51a  | 7.5 ± 1.08a  | 7.2 ± 0.78a  | 6.3 ± 1.25a  | 6 ± 0.00a    |
|                | T4         | 8.6 ± 0.51a | 8.2 ± 0.42ab | 7 ± 0.66a    | 7.1 ± 0.73a  | 6.5 ± 1.08a  | 5.9 ± 0.56a  |
|                | T5         | 8.5 ± 0.52a | 7.9 ± 0.73ab | 6.9 ± 0.87a  | 6.7 ± 0.67ab | 5.4 ± 0.51b  | 5.4 ± 0.51bc |
|                | T6         | 8.6 ± 0.51a | 7.9 ± 0.73ab | 7 ± 0.81a    | 6 ± 0.00b    | 5.5 ± 0.52b  | 5.4 ± 0.51bc |
4. Conclusions

The present investigation suggests an alternative method to prolong the shelf life of pomegranate arils during storage. The different formulations of chitosan (1%, 2%, or 3%) with the incorporation of CO (0.25% or 0.50%) were applied to the arils using a dipping method. The coating effect was observed on various physiological and biochemical parameters during cold storage (4 ± 1°C) up to 15 d. The results revealed that all treatments were found to be effective in improving the shelf life and postharvest characteristics of pomegranate arils. However, T3 treatment (chitosan 2% + CO 0.25%) had the highest potential to enhance the storage life quality attributes and reduce the microbial population of stored arils by 45%, followed by T4 treatment (chitosan 2% + CO 0.50%). Moreover, the complex coating formulations of chitosan-CO had the ability to control the microbial growth and the maintenance of the good sensory acceptability of arils during storage. Moreover, the treated arils exhibited excellent stability of physiological properties and antioxidant activity. Therefore, we conclude that the use of chitosan coating incorporated with cinnamon oil is a potentially adequate technique for maintaining the quality and extending the shelf life and biochemical components of pomegranate arils during cold storage.

Data Availability

Data are available upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

Mohit Singla and Sunil Pareek conceptualized, validated, and investigated the study. Mohit Singla, Nishant Kumar, and Sunil Pareek proposed methodology. Mohit Singla provided software and was responsible for formal analysis. Sunil Pareek contributed to resources, visualization, supervision, project administration, and funding acquisition. Mohit Singla, Nishant Kumar, and Narashans Alok Sagar were responsible for data curation. Mohit Singla and Nishant Kumar prepared original draft. Sunil Pareek, Narashans Alok Sagar, and Olaniyi Amos Fawole reviewed and edited the manuscript. All authors have read and agreed to the published version of the paper.

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<table>
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<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
<th>Day 15</th>
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<td>7.3 ± 0.67ab</td>
<td>6.7 ± 0.48a</td>
<td>6 ± 0.66b</td>
<td>5.6 ± 1.42b</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>7.9 ± 0.56a</td>
<td>7.5 ± 0.52ab</td>
<td>7 ± 0.81ab</td>
<td>6.9 ± 0.73a</td>
<td>6.8 ± 0.42a</td>
<td>5.6 ± 0.51b</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>8.4 ± 0.69a</td>
<td>8.0 ± 0.74a</td>
<td>7.8 ± 0.42a</td>
<td>7.2 ± 0.42a</td>
<td>6.7 ± 0.48a</td>
<td>6.4 ± 0.69a</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>8.1 ± 0.56a</td>
<td>7.7 ± 0.67ab</td>
<td>6.8 ± 1.03b</td>
<td>7 ± 0.66a</td>
<td>6.4 ± 0.69ab</td>
<td>5.9 ± 0.73b</td>
</tr>
<tr>
<td></td>
<td>T5</td>
<td>8.1 ± 0.66a</td>
<td>7.5 ± 0.52ab</td>
<td>7.3 ± 0.67ab</td>
<td>6.8 ± 0.78a</td>
<td>6.3 ± 0.48ab</td>
<td>5.6 ± 0.51a</td>
</tr>
<tr>
<td></td>
<td>T6</td>
<td>8.0 ± 0.62a</td>
<td>7.3 ± 0.48b</td>
<td>7.3 ± 0.48ab</td>
<td>6.7 ± 0.48a</td>
<td>6.1 ± 0.56b</td>
<td>5.5 ± 0.52a</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SD (n = 3) (P < 0.05). a, b, c, d, and e indicate significant differences in treatments compared to control with respect to an interval of 3 days (storage days 0, 3, 6, 9, 12, and 15 days, resp.). T0 = control, T1 = chitosan 1% + cinnamon oil 0.25%, T2 = chitosan 1% + cinnamon oil 0.50%, T3 = chitosan 2% + cinnamon oil 0.25%, T4 = chitosan 2% + cinnamon oil 0.50%, T5 = chitosan 3% + cinnamon oil 0.25%, and T6 = chitosan 3% + cinnamon oil 0.50%.

the present investigation. Aloe vera coating (100%) with acids (ascorbic and citric, 1%) also improved the sensory characteristics of arils than the control up to 8 d at 3°C storage [49].
References


