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# Smart edible coating films based on chitosan and beeswax–pollen grains for the postharvest preservation of Le Conte pear

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Smart edible coating films can be used in food packaging. For this application, they must have good physical and mechanical properties. Herein, chitosan–beeswax based film is used to preserve Le Conte pears postharvest. The chitosan–beeswax films were characterized using XRD, FT-IR, and SEM analysis. Moreover, all films showed good self-healing aptitudes ranging from 86.7 to 96.3. The film treated with pollen grains showed an enhanced water contact angle compared with the chitosan film. The chitosan–beeswax/pollen grain film exhibited a two-fold lower WVTR value compared to the chitosan film, and showed the tendency to increase the stiffness of the film. The elongation% at break was reduced from 35.81 to 14.09. Fruit quality parameters were determined in cold storage for 105 days during shelf life after a simulated marketing period of 7 days. All coated fruits successfully showed decrease in weight loss, decay and rate of softening. Therefore, chitosan–beeswax/pollen grains can be considered safe and effective coating for the fruit preservation.

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## 1. Introduction

Currently, biodegradable edible coating alternatives for post-harvest fruit preservation are attracting attention from researchers. Fortunately, smart edible coatings are one of these alternatives. Auto-healing coatings that can repair their damage automatically or by means of external stimuli have received attention recently.<sup>1–4</sup> Generally, self-healing materials include two main categories: inherent self-healing materials that exhibit dynamic reversibility of covalent and non-covalent bonds,<sup>5–8</sup> such as Schiff bases,<sup>9</sup> host–guest interaction,<sup>10</sup> Diels–Alder,<sup>11</sup> and hydrogen bonding,<sup>12</sup> and extrinsic self-healing materials that rely on the performance of agents encapsulated into hollow structures, microspheres, and nanospheres.<sup>13,14</sup> Inherent self-healing materials have aroused more interest than extrinsic ones because the latter frequently necessitate healing agents to repair the damage that occurs.<sup>15</sup>

Pear (*Pyrus communis*, L.) is one of the oldest plants grown in Egypt and is considered to be one of the most consumed fruits in the world. Its fruits are characterized by attractive flavor and aroma<sup>16</sup> as well as by high amounts of nutritional value, such as vitamins and minerals.<sup>17,18</sup> It is classified as a climacteric fruit, showing a peak in ethylene production and respiration rate during development. Hence, some deterioration is seen in the

color, appearance, firmness, sugar content and acidity.<sup>19</sup> Due to these factors, pear fruits are relatively perishable and difficult to maintain well for a longer period.<sup>20,21</sup> Accordingly, fruits suffer from luster loss, shrink and rotting due to relatively high water transpiration, nutrient loss and infection with microorganisms during storage.<sup>22</sup> Thus, in order to extend the shelf life of stored pear fruits, some technological advances must be applied to slow and limit the decline in fruit quality and generation of ethylene<sup>23</sup> as well as lengthen the postharvest life and maintain the quality of freshness.<sup>24</sup>

Edible coatings are non-toxic packaging materials that can regulate metabolic processes, improve the appearance of stored fruits, delay deterioration, and preserve product quality by extending the shelf life of fruits as well as ensure their brightness as an attractive factor for consumers.<sup>25–28</sup>

Recently, chitosan has received special attention in developing edible coatings due to its bioactivity, antimicrobial, and film forming capability.<sup>29–33</sup> Arnon *et al.*<sup>26</sup> fabricated a coating of carboxymethyl cellulose and chitosan layer by layer for the preservation of citrus fruits. Pollen grains are the major sources of protein, fats, vitamins and minerals.<sup>34</sup> Wax coatings create a modifying atmosphere around the fruit and provide a semi-permeable barrier for raising carbon dioxide levels and reducing oxygen levels, thereby reducing respiration, water loss, oxidation reaction rates and metabolic activities, especially respiration and transpiration. Therefore, coating fruits increases their resistance to pathogens and increases their storage and marketing capabilities.<sup>35,36</sup>

The main objective of this study was the preparation and characterization of a self-healing chitosan–beeswax-based film

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for maintaining quality criteria and extending the storage period of Le Conte pear fruits. Edible hydrophobic components, such as beeswax and pollen grains, were blended with chitosan to improve its physical and barrier properties while maintaining suitable mechanical characteristics. Three modifications were studied to reach these goals: beeswax–pollen grains, chitosan–glycerol/CaCl<sub>2</sub>·2H<sub>2</sub>O, and chitosan–beeswax/glycerol/CaCl<sub>2</sub>·2H<sub>2</sub>O/pollen grains, coded as (BW/PG), Chito and Chito–BW/PG, respectively, in comparison with water dipping as a control.

## 2. Experimental

### 2.1. Materials

**2.1.1. Pear orchard.** The target fruit sample of this investigation was Le Conte pear trees (*Pyrus communis*, L.). The trees were twenty years old, approximately uniform and vigorous, and budded on *Pyrus communis* root stock. They were developed in a loamy clay soil. The space was 5 × 5 m under a flood irrigation system. The healthy, almost uniform trees were almost identical in growth vigor and received the recommended horticultural treatments.

**2.1.2. Fruit sample.** Harvested mature fruits free from pathogen infection and blemishes at the beginning of August 2018 and 2019 were selected and delivered to the laboratory of Faculty of Agriculture Cairo University (Agriculture Development Systems (ADS) project). Chitosan (medium Mwt) was purchased from Sigma Aldrich. Beeswax (melting point 61–65 °C) was purchased from El Nasr Pharmaceutical Chemicals; CaCl<sub>2</sub>·2H<sub>2</sub>O was purchased from Loba Chemie.

### 2.2. Saponification and acid numbers of beeswax

The saponification and acid numbers of the beeswax were estimated according to the method reported by Elena *et al.*<sup>37</sup> The estimated saponification and acid number values were 542.75 mg KOH/g and 26.64 mg KOH/g, respectively.

### 2.3. Preparation of the self-healing chitosan–beeswax-based composite coating film

Firstly, beeswax pollen grain emulsion (BW/PG) was prepared by dissolving 0.5 g beeswax, 0.2 g glycerol, 0.5 g pollen grains and 0.2 g tween 80 in 100 mL hot distilled water. The whole mixture of the film-forming solution was homogenized at 70 °C for 2 minutes.

Secondly, the chitosan film-forming solution was prepared as described: chitosan (1 g) was dissolved in acetic acid (100 mL, 1%) and stirred at room temperature. After that, glycerol (0.2 g) and CaCl<sub>2</sub>·2H<sub>2</sub>O (30 mL, 0.4%) were added. Then, the solution was stirred for 30 minutes.

Then, the chitosan–beeswax/pollen grain coating composite was prepared, as described previously, in two steps: 0.5 g beeswax and 0.2 g glycerol, followed by 0.5 g pollen grains and 0.2 g tween 80, were added to the chitosan solution. The coating composite formulations were subjected to an ultrasonic bath to ensure complete removal of air bubbles before casting. The film-forming solutions were casted in Teflon casting plates. Then, the dry films were peeled off the plates and washed. The

films were desiccated at (20 ± 2 °C, 50% relative humidity) for one week to be ready for analysis.

### 2.4. Characterization of the self-healing chitosan–beeswax-based composite coating film

**2.4.1. Thickness measurements.** The thicknesses of the chitosan and chitosan–beeswax-based coating films were measured in accordance with the ISO 534-2005 protocol. A thickness gauge tester (Kalfer GmbH, Germany) accurate to 0.01 mm reading was used. Five replicates were measured per sample.

**2.4.2. X-ray diffraction (XRD).** The prepared composite materials were assessed *via* an X-ray Philips diffractometer (PW 1820 goniometer, PW 1930 generator), fixed with Cu K $\alpha$  radiation (45 kV, 40 mA, with  $\lambda = 0.15418$  nm). The analysis was scanned in a  $2\theta$  range of 5 to 60° with a step size of 0.02 and step time of 1 s.

**2.4.3. Fourier transform infrared spectroscopy (FT-IR).** The structures of the prepared composites were evaluated using a Bruker Vector 33 FTIR spectrometer (Germany). The infrared spectra were recorded in absorbance units in the 4000–400 cm<sup>-1</sup> range.

**2.4.4. Scanning electron microscopy (SEM).** The surface morphology investigation (SEM) of the chitosan–beeswax based coating film was carried out using an SEM (Quanta 250 FEG) attached with an EDX Unit, with accelerating voltage 30 kV., magnification 14× up to 1 000 000 and resolution for Gun.1n.

**2.4.5. Moisture content and degree of swelling%.** The moisture contents of the coating films were gravimetrically determined as clarified in eqn (1).<sup>38</sup> The degree of swelling was estimated by cutting the films into rectangular shapes (1 × 4 cm<sup>2</sup>,  $m_1$ ), which were dried at 105 °C for 24 h to achieve the dry mass ( $m_2$ ) as demonstrated in eqn (2). Then, the films were soaked in distilled water at 25 °C for 24 h to obtain a constant mass ( $m_3$ ). Lastly, the films were dried at 105 °C for 24 h ( $m_4$ ).

$$\text{Moisture content\%} = \frac{(m_1 - m_2)}{m_1} \quad (1)$$

$$\text{Degree of swelling\%} = \frac{(m_3 - m_2)}{m_2} \quad (2)$$

**2.4.6. Mechanical properties.** The mechanical properties (tensile strength and elongation% at break) were determined by ASTM D638-10.<sup>39</sup> All the studied dry films were cut into strips. Five strip samples of each film type were desiccated at 33% RH for 24 hours at ambient temperature. Two replicas of the coating films were cut into rectangular shapes (40 mm × 10 mm). The samples were clamped and subjected to tension until breakage. The strip samples were measured on an Instron Universal Testing Machine at a crosshead speed of 15 mm min<sup>-1</sup> and a 0.1 kN force load cell.<sup>40</sup>

**2.4.7. Self-healing efficacies%.** The ratio of healed stress or strain at the breaking point to the initial value is known as self-healing efficacy%, as illustrated in eqn (3). The coating films were cut into two identical pieces, and the film was left to heal under ambient temperature for the desired time. The fracture



stress or stain of the initial and healed specimens was determined.<sup>41</sup>

$$\text{Self-healing efficiency}\% = \frac{\text{stress heal or strain heal}}{\text{stress initial or strain heal}} \quad (3)$$

**2.4.8. Barrier properties (water vapor and oxygen transmission rate).** The water vapor transmission rate (WVTR) was determined using a GBI W303 (B) Water Vapor Permeability Analyzer (China) using the cup method. WVTR was measured as the mass of water vapor transmitted throughout a unit area in a unit time under controlled conditions of temperature (38 °C) and humidity (4%) according to a standard (ASTM E96). Also, the gas transmission rate (OTR) was measured by an N530 Gas Permeability Analyzer (China). According to the standard ASTM D1434-82 (2003).

**2.4.9. Water contact angle (WCA).** Herein, a Theta Optical Tensiometer (Model OAC, 13EC, DataPhysics Instruments GmbH, Germany) was utilized to detect the water contact angles (WCA). The single liquid dispenser (OAC 13 EC) dispensed a water drop with an exact volume of 1  $\mu\text{l}$ . It was inclined until the drop came in contact with the surface of the film. Then, the automatic dispenser was raised up with the end goal of retaining the water drop at the surface. Pictures were taken by a programmed camera. Then, the images were analyzed using the DataPhysics program, known as the Young fitting method, to perform the contact angle analyses.

## 2.5. Treatments and storage conditions

Two hundred and forty mature fruits were selected that were free from visual defects (“undamaged, uniform in shape, free from blemishes and pathogen infection”) and were divided into 4 treatment groups. The mature picked fruits were dipped in solutions containing beeswax–pollen grain, chitosan, and chitosan–beeswax/pollen grain coatings for 5 minutes in parallel to water as a control solution. Then, the coated fruits were left to air-dry. The treatment protocol included ten replicates per treatment and each replicate per 6 fruits; every treatment group contained approximately 60 fruits (12 kg), which were packed in fiberboard cartons under storing conditions ( $0 \pm 1$  °C and 85–90% RH) for 105 days. After fixed time intervals (15 days), the fruit samples were removed and fruit quality criteria estimations were carried out.

At the end of storage period, a sample (20 fruits) of each treatment group was left at room temperature ( $23 \pm 2$  °C and 47% RH) for one week as a marketing period, and quality assessments were performed.

## 2.6. Fruit quality assessments

**2.6.1. Physical characteristics.** The weight loss% of the all coated samples were estimated periodically by weight difference. The decay% was evaluated as follows: the decayed fruit samples were counted and discarded. The decay% was calculated based on the total numbers of the fruits. To determine the firmness (lb per inch<sup>2</sup>), on each sampling, 3 pear fruits from each treatment were analyzed for firmness using an Analogue

Portable Fruit Hardness Tester model FT 327 (3–27 lbs) equipped with a 8.0 mm cylindrical probe with a flat surface and height of 2 cm by measuring the penetration on both sides of the fruit of each of 3 fruits from the coated and uncoated groups in a vertical direction; then, the average was taken (6 readings per treatment).

**2.6.2. Chemical characteristics.** The total soluble solids percentages (TSS%) were determined using a hand refractometer. The total acidity (TA%) was estimated as the percentage of malic acid in fruit juice (AOAC, 2000).

The statistical analysis was performed using the computer-based software MS Excel, and the results were analyzed by variance.<sup>42</sup> LDS statistical analysis was used to define differences among treatment means at a significance level of 0.05.<sup>43</sup>

# 3. Results and discussion

## 3.1. The structure of the prepared chitosan–BW/PG film

FT-IR spectra of the BW, PG, CH, CH/BW and CH/BW-PG films are presented in Fig. 1. In the chitosan spectrum, a strong band at  $3247\text{ cm}^{-1}$  corresponds to N–H and O–H stretching, along with intramolecular hydrogen bonds. The absorption bands at  $2915$  and  $2848\text{ cm}^{-1}$  can be recognized as C–H symmetric and asymmetric stretching, respectively. The attendance of residual *N*-acetyl groups was verified by the bands near  $1607\text{ cm}^{-1}$  (C=O stretching of amide I) and  $1322\text{ cm}^{-1}$  (C–N stretching of amide III), separately. A band at  $1536\text{ cm}^{-1}$  indexes with the N–H bending of the primary amine.<sup>44</sup> The CH<sub>2</sub> bending and CH<sub>3</sub> symmetrical deformations were detected by the incidences of the bands at approximately  $1416$  and  $1380\text{ cm}^{-1}$ , respectively.<sup>45,46</sup>

The beeswax spectrum displayed noticeable infrared absorption peaks at  $3417$ ,  $2954$ ,  $2915$ ,  $2848$ ,  $1735$ ,  $1462$ ,  $1375$ ,  $1328$ ,  $1219$ ,  $1170$ ,  $729$ , and  $719\text{ cm}^{-1}$ . The peak at  $3417\text{ cm}^{-1}$  implies the existence of the stretching vibrations of hydroxyl groups of alcohols, the peaks at  $2915$  and  $2848\text{ cm}^{-1}$  point to the presence of fatty acid chains (caused by the stretching vibrations of C–H groups), the peak at  $1735\text{ cm}^{-1}$  is caused by the stretching vibrations of the carbonyls of esters, the peaks at  $2954$  and  $1462\text{ cm}^{-1}$  are caused by hydrocarbon vibrations, and the peaks at  $1328$ ,  $1219$ – $1170$  and  $729\text{ cm}^{-1}$  account for non-planar amide groups.<sup>47–49</sup> These results are similar to those of Dinker and Agarwal.<sup>50</sup> Intense absorption bands at  $2954$ ,  $2915$ ,  $2848$ ,  $1735$ ,  $1462$ ,  $1375$ ,  $1328$ ,  $1219$ ,  $1170$ ,  $729$ , and  $719\text{ cm}^{-1}$  appear in the FT-IR spectra of the chitosan/beeswax-based films, indicating the successful incorporation of beeswax into chitosan.

The FT-IR spectrum of the pollen grains shows an absorption band at  $1628\text{ cm}^{-1}$  (amide I: C=O stretch) of proteins, which interferes with the carbonyl groups in the chitosan/BW-PG film. The presence of aromatic rings was observed at the absorption band of  $1449\text{ cm}^{-1}$ . In the spectral region of  $1500$ – $866\text{ cm}^{-1}$ , a prominent band around the value of  $1375\text{ cm}^{-1}$  (COO-stretch and CH<sub>2</sub> and CH<sub>3</sub> deformation) appeared, which is attributed to the presence of lipids and triglycerides. The characteristic region of  $1500$ – $866\text{ cm}^{-1}$  that occurred in the FT-IR spectrum of the chitosan/BW-PG film indicates the presence of incorporated



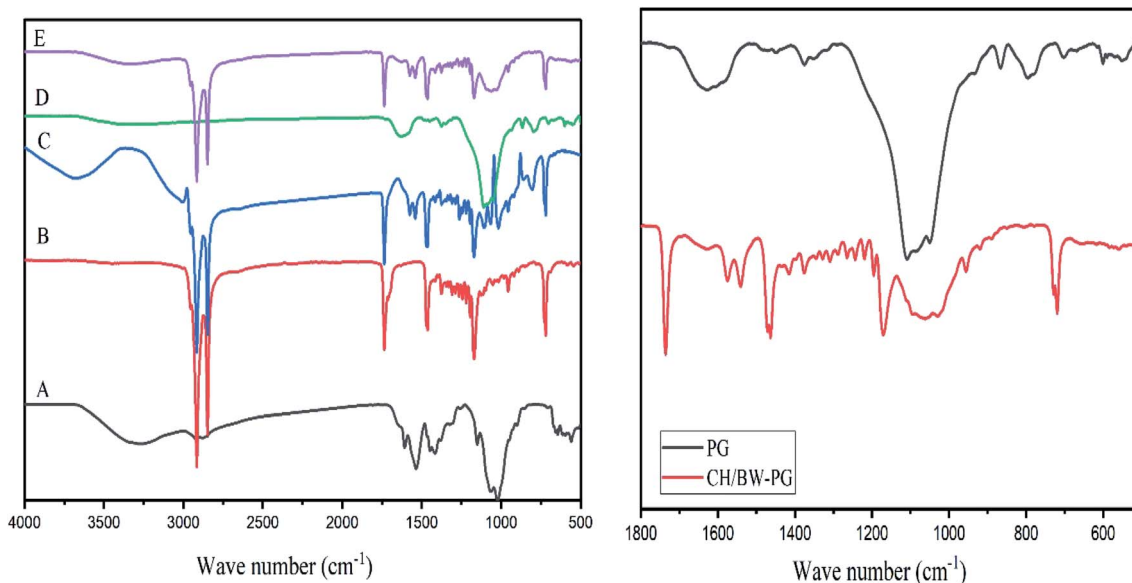


Fig. 1 FT-IR spectra of (A) CH, (B) BW, (C) CH-BW, (D) PG, and (E) CH/BW-PG.

pollen grains. The most intense band in this spectral region is at  $1109\text{ cm}^{-1}$  (C–OH skeletal; C–O–C). Carbohydrate molecules can also be observed around the band of  $866\text{ cm}^{-1}$  (C–O–C).<sup>51</sup>

### 3.2. X-Ray diffraction (XRD) pattern of the prepared chitosan–BW/PG film

XRD is an additional quick analytical practice for identification of the crystallinity of materials, and it is generally used for structural and compositional studies in material science. To support the FT-IR data and explore the crystallinity of the chitosan-based beeswax films, X-ray diffraction (XRD) analysis was executed on samples of natural beeswax, chitosan, pollen grains, and chitosan-based wax and pollen grain films. As shown in Fig. 2, the diffractogram of beeswax shows the seven

most intense peaks at  $2\theta = 10.3^\circ$  ( $d$ -spacing  $8.5979\text{ nm}$ ),  $21.53^\circ$  ( $d$ -spacing  $4.1232\text{ nm}$ ),  $23.90^\circ$  ( $d$ -spacing  $3.7199\text{ nm}$ ),  $29.98^\circ$  ( $d$ -spacing  $2.9785\text{ nm}$ ),  $36.11^\circ$  ( $d$ -spacing  $2.4849\text{ nm}$ ),  $40.31^\circ$  ( $d$ -spacing  $2.2354\text{ nm}$ ), and  $42.97^\circ$  ( $d$ -spacing  $2.1029\text{ nm}$ ), which are specific to the orthorhombic crystal structure in beeswax.<sup>52,53</sup>

The XRD analysis is indicative of the high purity and high crystallinity of the beeswax. As shown in Fig. 2, the diffractogram of chitosan shows two distinctive peaks appearing at  $2\theta = 12.13^\circ$  and  $22.10^\circ$ . These two peaks were observed in the diffractograms of the chitosan–BW and chitosan/BW-PG films with decreased intensities, indicating good incorporation of beeswax into the chitosan films. The pollen grains show five characteristic peaks observed at  $2\theta = 9.0^\circ$ ,  $21.0^\circ$ ,  $23.0^\circ$ ,  $38.39^\circ$  and  $44.62^\circ$ . In the diffractograms of the chitosan/BW-PG films, detectable pollen grain peaks were observed, but with small intensities; these peaks may be overlapped with the noise of the chitosan/BW-PG film.<sup>54</sup>

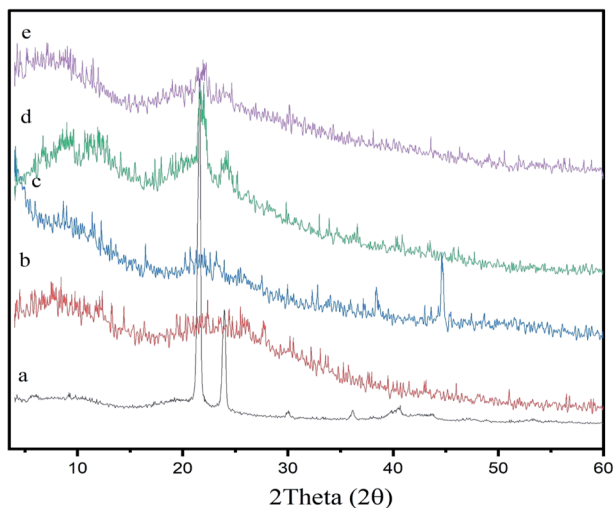


Fig. 2 XRD patterns of the (a) BW, (b) chitosan, (c) PG (d) chitosan–BW, and, (e) and chitosan/BW-PG films.

### 3.3. SEM images of the self-healing chitosan–beeswax films

The topographical and texture characterizations of the self-healing films comprising chitosan/beeswax/pollen grains (chitosan–BW/PG) cross-linked with  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  are shown in Fig. 3. The SEM images clarified that the chitosan–BW/PG film has a fairly rough surface morphology. These features of the surface morphology were probably affected by the crosslinking process, whereas the hydrogel has a fitted, rough, and dense structure, indicating good toughness and high surface area, which promotes its mechanical properties and water diffusion. The image showed uniform and homogeneous distribution of beeswax plus pollen grains with a lamellar appearance.

### 3.4. Contact angles of the chitosan–beeswax-based film

To predict the prospective interactions of the packaging materials with food, the water contact angle criterion was measured.



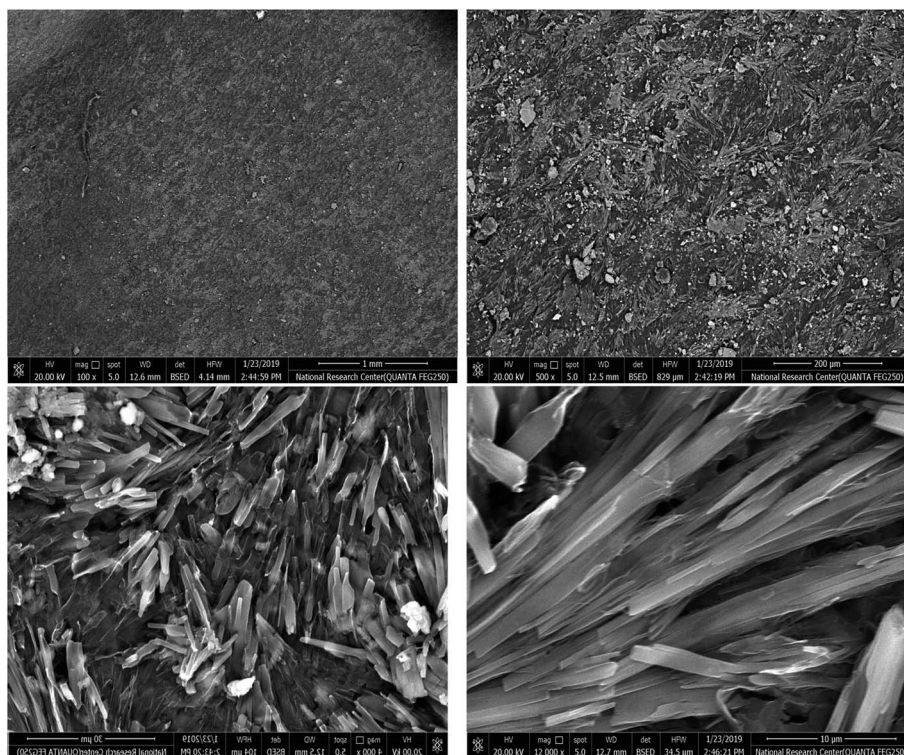


Fig. 3 SEM images of the self-healing chitosan–beeswax/pollen grains film at diverse magnification scales.

The water contact angles of the chitosan and chitosan–BW/PG films are presented in Fig. 4. The contact angle for the chitosan film is  $79.8^\circ$  because of the occurrence of hydroxyl groups in the chitosan/glycerol blend; therefore, the surface is partially hydrophobic. The contact angle value increased upon adding the beeswax and pollen grains to reach to  $109.7^\circ$ , which displays the enhancement of the hydrophobic character of the prepared film. Due to the surface hydrophobicity of the film, the spreading of water droplets was limited.

### 3.5. Physical and mechanical properties of the chitosan–beeswax-based composite film

Experimentally, beeswax/pollen grains have no capability to form well-matched coating films by normal casting procedures.

Thus, this formulation was used in a comparative study with the chitosan and chitosan–beeswax based coating films, as shown in Table 1.

### 3.6. Moisture content studies of the prepared chitosan–beeswax-based composite coating films

The moisture content of coating films governs the plasticizing parameter of these films. This leads to increased flexibility, and the produced films can resist cracking. The moisture content% was greatly affected by the presence of beeswax in the chitosan-based composites, as shown in Table 1. In all the chitosan-based composite formulations, the plasticizing agents were water and glycerol. The coating films containing beeswax have lower moisture contents than the chitosan coating films. This

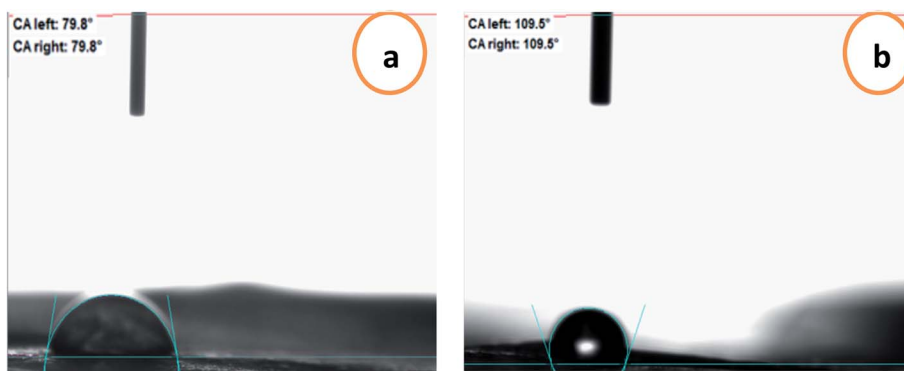


Fig. 4 Contact angle values of the chitosan (a) and chitosan–BW/PG (b) films.



Table 1 Physical and mechanical parameters of the self-healing chitosan–beeswax composite film

Test type	BW/PG	Chitosan	Chitosan–BW/PG
Thickness, ( $\mu\text{m}$ )	—	$34.00 \pm 1.50$	$38.9 \pm 1.60$
Moisture content, %	—	$26.44 \pm 0.02$	$25.44 \pm 0.03$
Degree of swelling, %	—	$2172.09 \pm 4.20$	$458.81 \pm 4.00$
WVTR, ( $\text{g per M}^2$ per day)	—	$2065.23 \pm 10.80$	$1038.07 \pm 5.78$
OTR, ( $\text{cm}^3$ per $\text{M}^2$ per day)	—	$0.034 \pm 0.004$	$0.13 \pm 0.03$
<b>CO<sub>2</sub>, GTR (<math>\text{cm}^3</math> per <math>\text{M}^2</math> per day)</b>	—	<b>10.40 <math>\pm</math> 0.25</b>	<b>25.85 <math>\pm</math> 0.45</b>
Tensile at break, (MPa)	—	$0.77 \pm 0.10$	$0.99 \pm 0.10$
Elongation at break, %	—	$35.81 \pm 0.30$	$14.09 \pm 0.27$
Young's modulus, (MPa)	—	$12.70 \pm 0.20$	$22.56 \pm 0.60$
Self-healing efficiency, %	—	$86.70 \pm 11.00$	$96.30 \pm 0.14$

behavior has been reported widely for films blended with beeswax. These results were reported by Elena Velickova *et al.*<sup>37</sup> Unlike other lipids, beeswax has noticeable resistance to moisture transport.

### 3.7. Swelling studies of the chitosan–beeswax-based composite coating film

The swelling degrees of the self-healing chitosan and chitosan–BW/PG-based composite coating films are mostly remarkable for postharvest preservation of fresh fruits. A self-healing chitosan–BW/PG-based film was fabricated by cross-linking with calcium chloride dihydrate. Generally, cross-linking leads to more reduction of the mesh size of the coating film, limiting the amount of water diffusion into the polymer matrix and cross-links.<sup>55</sup> The blending of beeswax and pollen grains into the chitosan formulation has a critical effect on the swelling degree% of the coating films. The chitosan formulation comprising beeswax has a reasonably lower swelling degree% than the chitosan coating formulation without beeswax. The swelling degree% of the self-healing chitosan–beeswax/PG-based film was  $458.81 \pm 4.0\%$  compared with that of the chitosan coating film ( $2172.09 \pm 4.2\%$ ), as shown in Table 1.

### 3.8. Permeability studies of the chitosan–beeswax based composite coating film

WVTR is a required element for appropriateness of packaging materials that are used for food packaging applications. Correspondingly, the path of water vapor (WV) from the neighboring air into food products or the loss of moisture from the foodstuff to the adjacent atmosphere meaningfully influences the stability and quality of the packaged food throughout the storage period. The barrier properties of the film contribute to giving a better indication of the applications of food packaging films, whereas the coating films are used to prevent dehydration of fruits.<sup>56</sup> The barrier properties of the self-healing chitosan and chitosan–beeswax/PG-based composite coating films are shown in Table 1. The existence of beeswax and pollen grains appears to increase the thickness of the coating films. The increasing thickness of the films may be due to the higher solid content. The beeswax is a key parameter that deeply affects the WVTR readings of the self-healing chitosan and chitosan–

beeswax/PG-based films, which resulted in a sharp decrease in WVTR values compared with the chitosan formulation without beeswax and pollen grains. The self-healing chitosan–beeswax/PG-based composites exhibited a lower WVTR value of up to  $1038.07 \pm 5.78$  g per  $\text{m}^2$  per day compared with that of the chitosan coating film ( $2065.23 \pm 10.8$  g per  $\text{m}^2$  per day).

Moreover, the oxygen transmission rates (GTR) of the self-healing chitosan and chitosan–beeswax/PG based films were generally markedly low. This parameter is probably related to the nature of the coating film, and the permeate (oxygen gas) affects its transfer through the film.<sup>57</sup> This is because polar molecules diffuse faster than non-polar molecules, especially through polar films. This is due to the increased solubility of polar molecules in polar films. It is clear that the self-healing chitosan–beeswax/PG-based coating film exhibited higher GTR than the chitosan film. The GTR value for the self-healing chitosan–beeswax/PG-based coating films was detected and compared to that of the chitosan coating film, reaching values as high as  $0.13 \pm 0.033$   $\text{cm}^3$  per  $\text{M}^2$  per day compared with chitosan ( $0.034 \pm 0.004$   $\text{cm}^3$  per  $\text{M}^2$  per day). The addition of the highly hydrophobic beeswax as a component of the coating composite is responsible for the increasing film hydrophobicity; also, the oxygen gas diffusion increased, and thus the GTR increased. Also, Table 1 shows that the GTR of carbon dioxide (CO<sub>2</sub>) of the fabricated chitosan–beeswax/PG based composites improves upon loading beeswax/PG in the chitosan–beeswax/PG composites. This is owing to the specific structures of beeswax/PG in the prepared composite. Therefore, the permeability of CO<sub>2</sub> of the prepared chitosan–beeswax/PG based composites was enhanced from  $10.40 \pm 0.25$  to  $25.85$  ( $\text{cm}^3$  per  $\text{M}^2$  per day) compared with chitosan.

### 3.9. Mechanical properties and self-healing efficiency%

The incorporation of long chain hydrocarbon beeswax into self-healing chitosan-based composite films creates a considerably less flexible coating film. The reduction in film elasticity may be a result of the lower absorbed water content in the self-healing chitosan composites containing beeswax.<sup>58</sup> The increased toughness of the films is evidenced by the increasing Young's modulus, tensile strength, and reduced elongation% at break, as demonstrated in Table 1. The chitosan–BW/PG film is a considerably less flexible film. A two-fold decrease of the



elongation% at break was observed for the chitosan–BW/PG film ( $14.09 \pm 0.266\%$ ) compared with the chitosan film ( $35.81 \pm 0.3\%$ ). Also, the Young's modulus showed a twofold increase for the chitosan–BW/PG film ( $22.56 \pm 0.6$  MPa) compared with the chitosan film ( $12.7 \pm 0.2$  MPa). The chitosan and chitosan–beeswax coating films exhibit perfect self-healing modes; they tend to auto-heal under the ambient temperature without any external effects.

The self-healing efficiency% is presented in Table 1. The results indicated that the PEM-SH coating films have high self-healing efficiency% within the range of  $83.03 \pm 9.3\%$  to  $92.00 \pm 1.9\%$ . The real reasons for the auto-healing mode of the films may be dynamic supramolecular interactions that comprise electrostatic interactions and hydrogen bonding. In our work, the auto-healing aspects may be due to presence of  $\text{Ca}^+$  ions, which diffuse along the interface of the cut points and tend to re-build the supramolecular interactions at the connection points. Due to the inherently dynamic nature of the polymer, the hydrogen bonds would be re-constructed again between the two cut fragments.<sup>59</sup>

### 3.10. Fruit quality assessments after storage

#### 3.10.1. Fruit physical characteristics

**3.10.1.1. Fruit weight loss percentage.** The data in Fig. 5(a and b) show that the weight loss (%) of the fruits shows a slight but significant increase as the storage periods progress, ranging

from 0.20 to 7.10 in the 1<sup>st</sup> season and from 0.56 to 7.97 in the 2<sup>nd</sup> season. These findings suggest that all the coated samples have a high tendency to reduce the weight loss percentage of Le Conte pear fruits compared with that of uncoated fruits under cold storage conditions. The most efficient coating treatment is BW/PG, followed by chitosan and chitosan–BW/PG, respectively, compared with the control; the trend exhibits a descending order, but there is no significant difference among them. The actual reasons for these results may be water evaporation, transpiration and dry matter by respiration. Using the edible coatings may create a thin film that surrounds the fruit peel and induces a modification of the atmosphere around the fruits. The film is semipermeable against  $\text{CO}_2$ ,  $\text{O}_2$ , water vapor molecules and solute transport. Therefore, the water loss, oxidation and respiration rates would be reduced.<sup>60</sup>

Comparable results were reported for previous studies on beeswax alone by Bashir and Abu-Goukh<sup>61</sup> in guava by and El-Anany *et al.*<sup>62</sup> in Anna apple, for chitosan alone by Kerch<sup>63</sup> in several fruits and vegetables and for combined coats (beeswax + chitosan) by Asgar *et al.*<sup>64</sup> on papaya fruits. The weight loss criterion is an important issue to consumers. Therefore, the use of edible coatings is considered to be an excellent option to limit weight loss.<sup>65</sup>

**3.10.1.2. Fruit decay percentage.** Looking at Fig. 6(a and b), it is obvious that the fruit decay% increased steadily and gradually as the storage period progressed up to 105 days at  $0 \pm 1^\circ\text{C}$  in

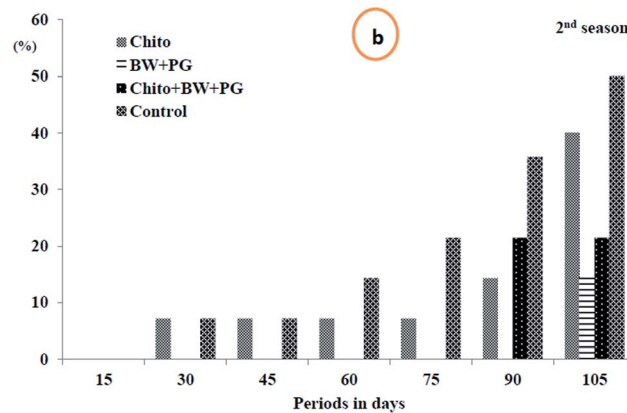
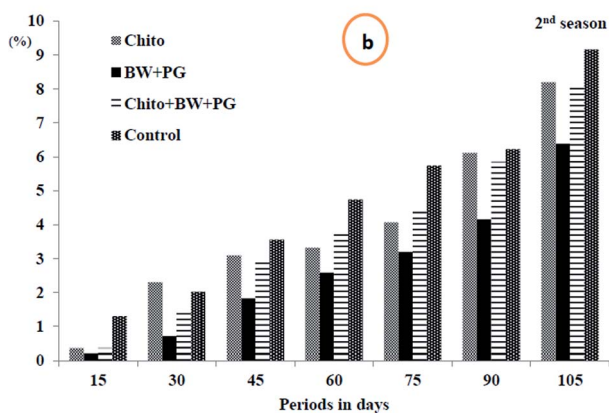
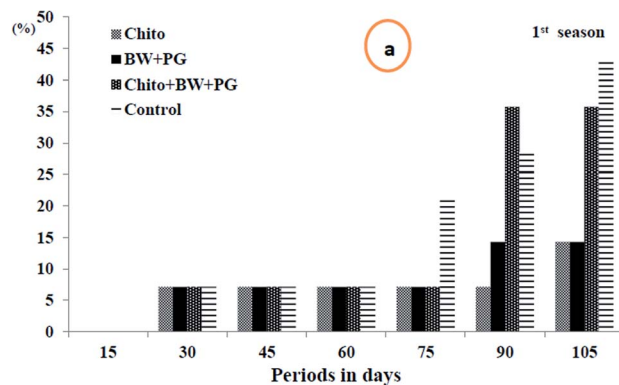
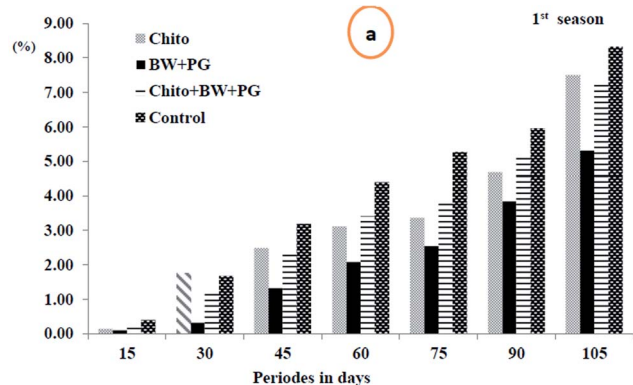


Fig. 5 (a and b): Weight loss% of pear fruits coated with chitosan (Chito), beeswax/pollen grains (BW + PG), chitosan/beeswax/pollen grains (Chito + BW + PG) and the control sample (water) in the first and second seasons.

Fig. 6 (a and b): Decay% of pear fruits coated with chitosan (Chito), beeswax/pollen grains (BW + PG), chitosan/beeswax/pollen grains (Chito + BW + PG), and the control sample (water) in the first and second seasons.



a significant way in the two seasons, except that this increment was fixed up to 60 days in the first season and up to 45 days in the second season. In both seasons, after 15 days of storage, all the studied samples exhibited fruits that were healthy and free from decay, in accordance with the interaction between different treatments and storage periods. Although in the 1<sup>st</sup> season, the fruit decay% was steady up to 60 days (7.15%) in all treatment groups, it continued steady up to 75 days in both treatment groups (BW/PG) and chitosan-BW/PG, which recorded 7.15%, and in fruit coated with chitosan up to 90 days, which reached 7.15%. Meanwhile, in the second season, the fruit decay% was fixed for up to 45 days in uncoated fruits and up to 75 days in fruits coated with chitosan, which recorded 7.15%. Meanwhile, fruit coated with chitosan-BW/PG did not decay up to 75 days; however, fruits coated with BW/PG remained healthy and free of decay for up to 90 days. The highest significant% of decay was obtained at 105 days in uncoated fruits (42.90% and 50.05% sequentially in the two seasons).

Regarding the effect of treatments on decay%, the lowest values of decay were obtained from fruits coated with chitosan, which recorded (7.15%), followed by fruits coated by BW/PG (8.17), then chitosan-BW/PG-coated fruits (14.3%) in the 1<sup>st</sup> season. Otherwise, lower decay% were revealed from fruits coated with BW/PG (2.04%), chitosan-BW/PG (6.13%) and chitosan (11.85%), respectively, in the 2<sup>nd</sup> season. The highest decay% was shown by the uncoated fruits (16.34% and 19.41%)

consecutively in both seasons, and no significant differences were detected between all treatments in the two seasons. The reduction in fruit decay% by the application of an edible coating was due to the partial restriction of gas exchange through the peel; also, later, ethylene gas generation was inhibited. Due to this inhibitory mechanism, the postharvest fruits are more resistant to decay. The water loss may cause minor wounds on the peel, and in that way, the decay% will be reduced. Owing to the off-flavor which results from fruit rotting,<sup>66</sup> in other studies, chitosan has been used extensively as an inhibiting elicitor for postharvest senescence and diseases in many fruits, such as pear, citrus, kiwifruit, apple peach, strawberry and sweet cherry.<sup>63</sup> These results were in agreement with Ahmed *et al.*;<sup>67</sup> they mentioned that treatment with chitosan has the potential to inhibit decay and hence prolong the cold storage life of navel orange fruits.

**3.10.1.3. Fruit firmness (lb per inch<sup>2</sup>).** Fruit firmness is one of the most important physical considerations to evaluate the progress of ripening. Fruit firmness is an indicator of ripeness degree because it is recognized as the force required to break the flesh tissues. It is associated with various ripening stages. The data in Fig. 7(a and b) display clearly that the flesh firmness of pear fruits significantly decreased with advancing storage period for all treatments during cold storage at  $0 \pm 1$  °C, and the minimum was revealed at up to 105 days, which was recorded as 11.73 and 11.34 lb per inch<sup>2</sup>, respectively, in both seasons. Coated pear fruits showed higher effects in reducing the rate of fruit softening. The combined coated fruits with chitosan-BW/

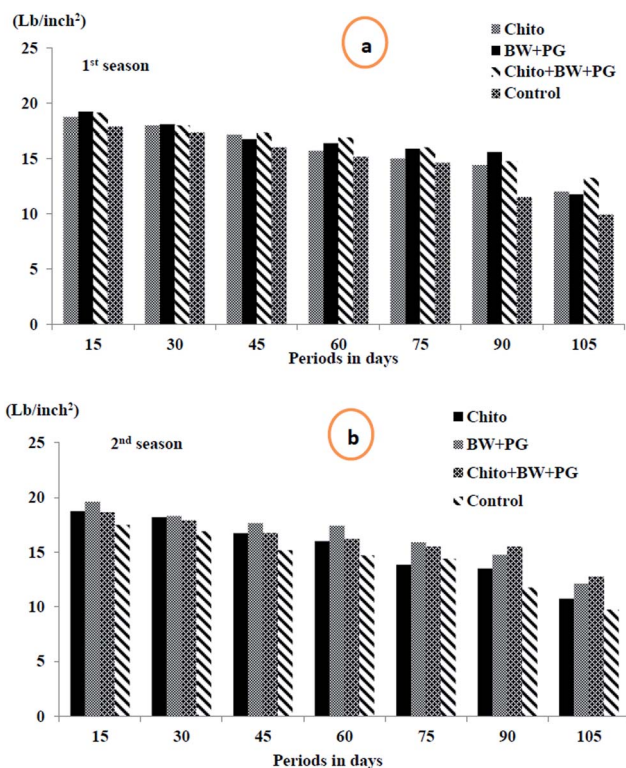


Fig. 7 (a and b): Firmness (lb per inch<sup>2</sup>) of pear fruits coated with chitosan (Chito), beeswax/pollen grains (BW + PG), chitosan/beeswax/pollen grains (Chito + BW + PG) and control sample (water) in the first and second seasons.

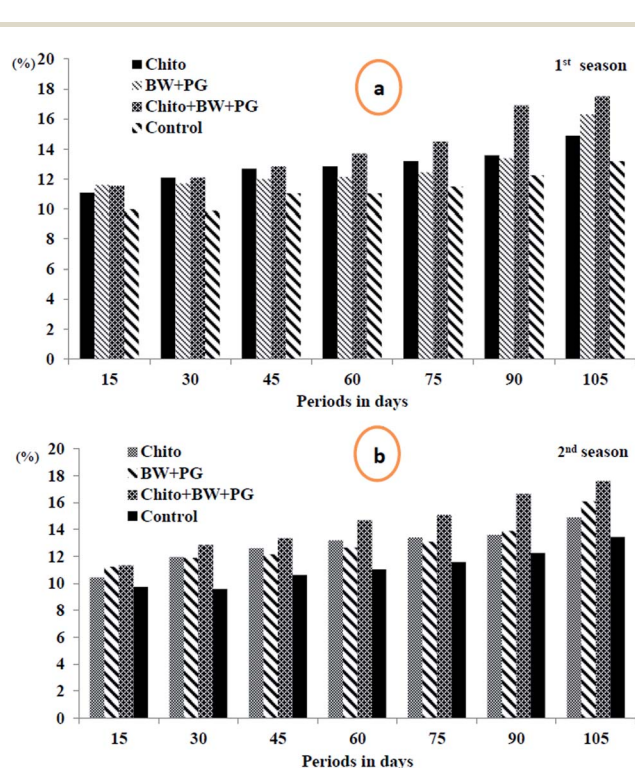


Fig. 8 (a and b): TSS% of pear fruits coated with chitosan (Chito), beeswax/pollen grains (BW + PG), chitosan/beeswax/pollen grains (Chito + BW + PG) and the control sample (water) in the first and second seasons.







Fig. 9 (a and b): TA% of pear fruits coated with chitosan (Chito), beeswax/pollen grains (BW + PG), chitosan/beeswax/pollen grains (Chito + BW + PG) and the control sample (water) in the first and second seasons.

PG achieved low softness after up to 105 days of cold storage (13.25 and 12.75 lb per inch<sup>2</sup>), followed in the same period by BW + PG-coated fruit (11.75 and 12.10 lb per inch<sup>2</sup>), sequentially, in the two seasons. Chitosan-coated fruit came next in this respect. The control fruits showed more significant softness than those with the other treatments.

Pears ripened to a soft melting texture are considered 'ready to eat'. As with other fruit species, the cell wall extracted from pear fruit showed considerable increase in swelling and high pectin solubilization during ripening. This phenomenon can be explained by previous research that indicated that disassembly of the primary cell wall and middle lamella structures of fruit flesh could contribute to changes in fruit texture during storage. Firmness during ripening in climacteric fruit, such as pears, is generally attributed to degradation of the cell wall and loss of turgor pressure in the cells reduced by water loss.<sup>68</sup> The reduction in fruit softness by application of an edible coating (wax and/or chitosan coating alone or combined) may be due to the inhibition of water loss and the activities of pectin-degrading enzymes closely related to fruit softening by reducing the rate of metabolic processes during senescence. These results are similar to those of other authors for the preservation of different varieties of pears, such as "Flor de Invierno",<sup>69</sup> "Huanghua",<sup>70</sup> "d'Anjou"<sup>71</sup> and "Le Conte".<sup>66</sup>

### 3.10.2. Physical-chemical characteristics of the fruit

**3.10.2.1. Total soluble solids percentage (TSS%).** The results in Fig. 8(a and b) indicate that the TSS% increased significantly throughout the progress of the storage periods at  $0 \pm 1$  °C, and the maximum was obtained for up to 105 days by all treatments

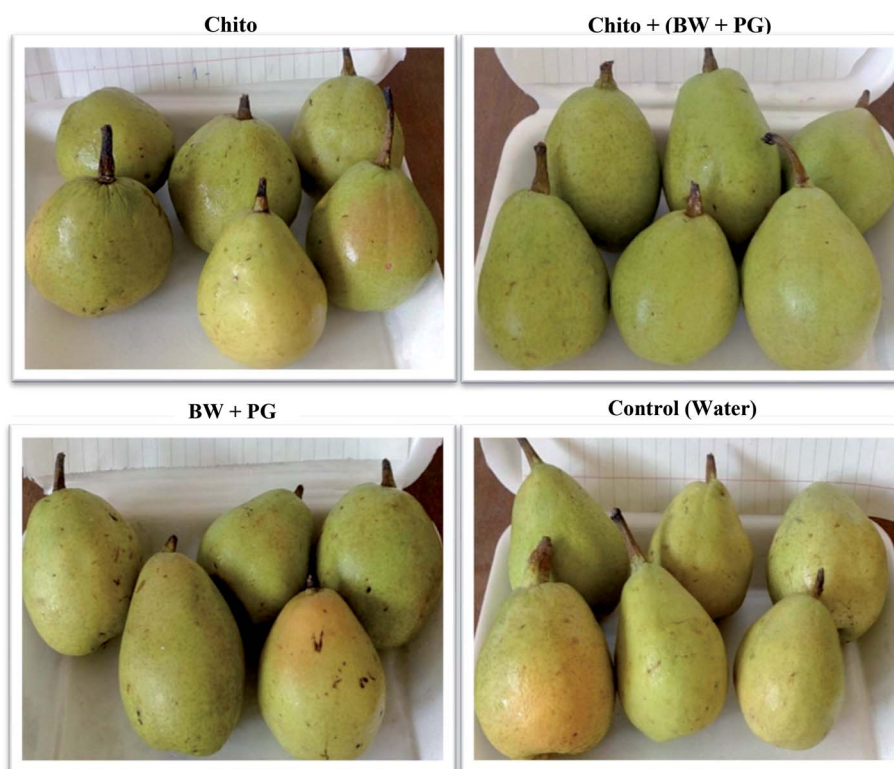


Fig. 10 Effects of BW/PG, chitosan and chitosan-BW/PG on the appearance of Le Conte pear fruits stored at  $0 \pm 1$  °C for 105 days + one week at  $23 \pm 2$  °C as a simulated marketing period during the 2018 and 2019 seasons.



in the two seasons. All the coated fruits showed positive increases in TSS% when compared with uncoated fruits. The best treatment in this regard was fruit coated with chitosan-BW/PG, which was more effective statistically in increasing TSS% in both seasons. This was followed by chitosan-coated fruit in the 1<sup>st</sup> season, in contrast with BW/PG-coated fruit in the 2<sup>nd</sup> season. The lowest significant levels of TSS% were detected for the uncoated fruits in both seasons.

The increase in total soluble solids in fruits is directly correlated to the hydrolytic activities of starch, the increased activity of enzymes responsible for the hydrolysis of starch to soluble sugars, and the conversion of starch to sugar, which indicates that the fruits are in the ripening process.<sup>66</sup> Similar findings were observed in some fruits treated with edible coatings, such as those reported previously for Raafat *et al.*<sup>25</sup>

**3.10.2.2. Total acidity percentage (TA%).** The results in Fig. 9 demonstrate that there was a gradual decline in the fruit juice TA% of Le Conte pear with advancing storage period in cold storage up to 75 days, followed by a slight increase up to 105 days, in the two seasons. Fruits coated with chitosan-BW/PG stored for 15 days scored the highest values of this parameter compared with the other different storage periods in both seasons. In general, all pear fruits, coated or uncoated, gave values of TA% with no significant differences among them in both seasons. Fruit acidity is another important factor affecting consumer acceptance. The main acid present in pear is malic acid, and its level decreases during ripening. As the ripening of the fruits develops, a reduction in titratable acidity is observed. The decrease in acid content is caused by the use of acids in the fruit as a source of energy and the conversion of organic acids to form sugar.<sup>72</sup>

The reduction in TA% of the coated fruits may be due to a decrease in the respiration rate, while the increasing TA% at the end of the storage periods may be due to absorption of

amino acids and their transformation to organic acids throughout metabolic activities, especially respiration and transpiration. Our results are in harmony with those obtained by Kou *et al.*<sup>73</sup>

### 3.11. Fruit quality assessments after shelf life (marketing period)

The data in Table 2 demonstrate that all test treatments in this study showed approximately the same trend of the influence on the physical and chemical characteristics of Le Conte fruits throughout cold storage ( $0 \pm 1$  °C) for 105 days + 7 days at room temperature ( $23 \pm 2$  °C) as a simulated marketing period (shelf-life), and this was confirmed in both seasons. Moreover, all edible coatings were more effective in improving Le Conte fruit quality when compared with uncoated fruits. The Chitosan-BW/PG-coated fruits were more pronounced in reducing their percentages of weight loss (0.80% and 3.13%) and decay (0.0% and 10.0%) as well as progress in increasing firmness (11.75 and 12.35 lb per inch<sup>2</sup>) and TSS% (11.25% and 11.65%) in the 1<sup>st</sup> and the 2<sup>nd</sup> seasons, respectively.

The fruits coated only with BW/PG and chitosan came next in this regard. Concerning the effects of these coating treatments on TA%, the fruits coated with combined chitosan-BW/PG (0.37% and 0.37%) came after those coated with chitosan alone (0.25% and 0.25%), BW/PG (0.32 and 0.32) and the control (0.32% and 0.32%), respectively, with little change. In contrast, the control treatment obtained the highest significant values of weight loss (3.89% and 6.51%) and decay (40.0% and 40.0%) and also recorded the lowest significant values of firmness (8.50 and 8.50 lb per inch<sup>2</sup>) and total soluble solids (9.10% and 9.60%), sequentially, in both seasons.

The above results are in line with findings by Virgilio *et al.*<sup>66</sup> that showed that the application of an edible coating (wax) will

Table 2 Effects of BW/PG, chitosan and chitosan-BW/PG on quality assessments of Le Conte pear fruits stored at  $0 \pm 1$  °C for 105 days + one week at  $23 \pm 2$  °C during the 2018 and 2019 seasons

Treatments	Physical characteristics					
	Weight loss (%)		Decay (%)		Firmness (lb per inch <sup>2</sup> )	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Chitosan	2.03	3.66	10.00	20.00	10.75	11.00
BWPG	1.81	3.19	10.00	20.00	11.10	11.75
Chitosan-BW/PG	0.80	3.13	00.00	10.00	11.75	12.35
Control (water)	3.89	6.51	40.00	40.00	8.50	8.50
<b>LSD<sub>0.05</sub></b>	<b>0.14</b>	<b>0.1</b>	<b>10.90</b>	<b>7.80</b>	<b>0.13</b>	<b>0.83</b>
Treatments	Chemical characteristics					
	Total soluble solids (%)		Total acidity (%)			
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season		
Chitosan	11.10	11.20	0.25	0.25		
BW/PG	11.20	11.45	0.32	0.32		
Chitosan-BW/PG	11.25	11.65	0.37	0.37		
Control (water)	9.10	9.60	0.32	0.32		
<b>LSD<sub>0.05</sub></b>	<b>0.50</b>	<b>0.39</b>	<b>0.03</b>	<b>0.07</b>		



partially restrict gas exchange through the fruit peel and inhibit the action of ethylene. This inhibitory action can provide better protection against postharvest decay in fruits. In addition, the water loss may cure minor wounds on the peel of the fruits and thereby reduce fruit rot, which results in the development of off-flavour. The influence on the respiration rate and ethylene by the coatings is specific to each fruit and variety. Thus, these parameters are important when coating and packaging are studied postharvest.

Coating is therefore necessary for storage and marketing. Also, Yage *et al.*<sup>74</sup> indicated that a chitosan coating is beneficial to maintain the storage quality and prolong the shelf life of post-harvest fruits; it is always used as the carrier film for antimicrobial agents. Moreover, Ahmed *et al.*<sup>67</sup> demonstrated that treatment with chitosan prolongs postharvest shelf life during shipping and marketing and is also safe for human health and the environment. Fig. 10 displays that the chitosan-BW/PG-coated fruit showed the best parameter values to preserve the fruits (better appearance, bright, attractive, less weight loss and decay, high firmness and TSS); thus, it is the best treatment to improve the quality and prolong the shelf life and marketing period of Le Conte pears. The previous results are in harmony with the findings of Gol *et al.*,<sup>75</sup> Kerch,<sup>63</sup> Virgilio *et al.*<sup>66</sup> and Nair *et al.*<sup>76</sup>

## 4. Conclusions

Smart biodegradable and edible coatings may be good alternative candidates for postharvest preservation of fruits. Opportunely, inherently auto-healing edible coatings can autonomously re-build their integral networks. Our observations in this study demonstrated that coating fruit with combined chitosan-BW/PG was the best treatment to maintain the quality of Le Conte pears during storage life and marketing periods. Thus, it is considered to be a safe and effective method as well as an environmentally friendly technology that is safe for human health.

## Conflicts of interest

There are no conflicts to declare.

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