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Integrating waste fish scale-derived gelatin and chitosan into edible nanocomposite film for perishable fruits

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ABSTRACT

Petroleum-based plastics (such as polyethylene, polypropylene, polyvinyl chloride, polystyrene, etc.) as white waste have caused great concern in the environment. It is urgent to develop a kind of biodegradable, biocompatible and non-toxic materials to replace them. Herein, an environmental-friendly edible film for postharvest fruits refreshing application was prepared by combining the waste fish scale-derived gelatin, chitosan as well as $CaCO_3$ nanoparticles. The as-prepared nanocomposite film showed the multifunctional features, such as UV absorption, antimicrobial, oxygen screening, excellent mechanical properties and non-toxic. In addition, the protein-polysaccharide based nanocomposite film was hydrophilic and can be easily washed away on fruits before eating. In order to inspect its preservative effect on fruits, longan and banana were chosen as the testing object. Our results showed that the edible multifunctional nanocomposite film can effectively extend the shelf life of longan by more than 3 days and banana by more than 5 days, compared with the control groups. Integrating natural biological macromolecules gelatin and chitosan into a multifunctional nanocomposite film with series of advantages of biodegradability, sustainability as well as multifunction is expected to be a potential preservative material for food packaging applications.

1. Introduction

Since the strict plastic-banned order has been issued in China in January 2021, it is an urgent problem to develop the degradable and sustainable materials instead of plastics for various applications, such as food packaging or freshness. Compared with petroleum-based plastics (such as polyethylene, polypropylene, polyvinyl chloride, polystyrene, etc.), nature-derived biopolymers such as proteins, polysaccharides and lipids have attracted much attention for formation of edible films and coatings because of their unique properties, such as sustainability, biodegradability, as well as nutrition value [1,2]. Among them, gelatin as a kind of important biological macromolecular, which has the advantages of colorless, tasteless, translucent, film forming ability, UV resistance and emulsification characters is frequently employed as the resource for the preparation of the edible film [3]. However, the use of gelatin as the raw material of edible film in food preservation will face the problems of poor mechanical strength, weak moisture resistance and stability, etc. [4]. Combining gelatin with other types of polysaccharides (such as chitosan, pectin) might be a good way to solve these problems. In addition, it is also of great importance to modify the formulation of the gelatin-based complex edible film to make it more versatile, such as antibacterial, isolation of oxygen, increasing mechanical properties and so on, the aim to lengthen the shelf life of fresh goods or processed foods.

However, gelatin is short of antimicrobial activities, which are of prime importance during food preservation [5]. To this end, a kind of natural polysaccharide-chitosan with biocompatibility, biodegradation,

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non-toxicity, is usually selected as auxiliary to mix with gelatin together [6,7]. This is because that chitosan not only has an outstanding inhibitory effect on fungi and bacteria, but possesses an excellent film-forming and moisture resistance abilities [8]. Thus, chitosan is an ideal candidate to fabricate fish scale-based edible film. Despite all these advantages, the as-prepared film still undergoes an unsatisfying mechanical strength. Inspired by the natural biomaterials (such as shell, bone, tooth) with elaborate structures and excellent mechanical performances, forming organic-inorganic hybrid might be a good approach to solve this problem. For example, abalone shell (mainly is made up of nano-$CaCO_3$), has prominent mechanical properties, such as the coexistence of high strength and toughness [9]. It is generally believed that the mineral component nano-$CaCO_3$ is responsible for the enhanced strength of such kind of hybrids. Moreover, some studies have shown that nanoparticles can improve the mechanical properties of the film [10]. As well known, $CaCO_3$ is a kind of important additive in industry, and widely used in plastics, rubber, and papermaking to enhance their mechanical strength and reduce the cost [11]. In addition, $CaCO_3$ is also used as essential Ca resources in food industry to supplement nutritional components for bone growth [12].

Gelatin used is usually derived from the skin or bones of animals, whereas it might spread infectious spongy tissue brain disease (foot-and-mouth disease and prions) [13–15]. Therefore, its' safety has aroused great concern. Some countries in Europe have banned the use of gelatin extracted from land mammals for medicine and food [16]. Since the relatives of fish and human are far away, there is little concern about zoonotic disease for fish. Fish scale gelatin (FSG) is an environmentally friendly and abundant natural biological resource, and also contains some nutrition [17–19]. China has become the world's largest aquaculture producer, thus is inexpensive and plentiful [20]. However, most fish scale is abandoned as waste. If we can fully explore fish scale as the resource of edible film, it will not only turn the "waste into treasure", but also endow the resulting product with health value (such as the ability of collagen to improve wound healing and reduce scar formation. Inhibit lipid oxidation and prevent food spoilage. An emerging application of marine gelatin from the recent discovery of their implication in the regulation of the sense of satiety, fat metabolism and 2 diabetes) [21–24].

In this work, gelatin was extracted from waste fish scales, then was plasticized with glycerin, afterwards chitosan was added to endow the film with extra antibacterial and film-forming ability, and then, nano-$CaCO_3$ was synthesized by means of a biomineralization method. The main novelty of this study is that the nano-$CaCO_3$ can improve the mechanical properties of the edible film. These biofilms have the potential to use as antibacterial and edible coating for fruits, and they also can be used for eco-friendly packaging applications. The nanocomposite film has the ability to protect the fruits against UV, spoilage and microbes, so they can keep them fresher in an eco-friendly sustainable way and reduce the fruit waste.

2. Material and methods

2.1. Materials and reagents

Fish scales were provided by Haixin Food Co., Ltd., Shishi, Quanzhou, China. Chitosan (deacetylation degree is 80.0–95.0%) and glycerin were purchased from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China). Acetic acid (100%) and hydrochloric acid were purchased from Xilong Chemical Co., Ltd. (Guangzhou, China). Ultrapure water was used as the solvent during the entire research process (Xi'an YouPu Instrument and Equipment Co., Ltd., Xi'an, China).

2.2. Film preparation

2.2.1. Fish scale gelatin (FSG) film preparation

Fish scale was washed with water to get rid of impurities and dried. The fish scale (20 g) was treated by 0.6 M hydrochloric acid, and then washed with distilled water until neutral. The treated fish scales were kept at 95 °C for 3 h. After cooling and centrifugation (8000 rpm), the supernatant (fish scale gelatin) was used as film-forming solution. 2% (v/v) of glycerin was added to the film-forming solution (FSG) and stirred evenly. The above FSG solution was poured into a petri dish, and dried (the film was used air conditioning wind at 26 °C for 48 h, the wind speed is mid-range). After drying (the film was stored at 26 ± 1 °C, RH = 50%), the formed FSG film was peeled off for further characterization.

2.2.2. Preparation of FSG&$CaCO_3$ film

$CaCl_2$ (0.5 M) and Na_2CO_3 (0.5 M) were used as raw materials to generate $CaCO_3$ nanoparticles directly in FSG [25]. 1 mL Na_2CO_3 solution was mixed with the solution of 1 mL $CaCl_2$ and 10 mL FSG solution under stirring. The above solution was poured into a petri dish, and dried. After drying, the formed FSG&$CaCO_3$ film was peeled off for further characterization (the method is the same as FSG film).

2.2.3. Preparation of FSG&$CaCO_3$&Chitosan film (the edible multifunctional nanocomposite film)

Firstly, chitosan (2% w/v) was dissolved with acetic acid (1% v/v). Whereafter, chitosan solution was mixed with FSG&$CaCO_3$ solution in equal volumes to form the edible multifunctional nanocomposite solution, which was used for the longan and banana preservation experiment. The above solution was poured into a petri dish, and dried. After drying, the formed FSG&$CaCO_3$&Chitosan film (the edible multifunctional nanocomposite film) was peeled off for further characterization (the method is the same as FSG film).

2.3. Characterization

The surface and cross-section of the film was observed by the scanning electron microscope with in situ elemental analysis via X-ray spectrometry (EDX) (SEM, Zeiss, Germany), Fourier-Transform Infrared Spectroscopy (FTIR) was recorded by Nicolet iS50 FT-IR (Thermo Scientific, USA) in the range of 4000–650 cm^{-1}. The $CaCO_3$ obtained in the film was analyzed by X-ray diffractometer (XRD, Bruker D8 Advance, Germany) radiated by Cu Kα, radiation operating at 40 kV and 40 mA. The diffraction patterns were recorded in the 2θ range of 10–80° with a scanning rare of 10° min^{-1}. The visible and ultraviolet (UV) light barrier properties of the film were measured barrier properties (200–600 nm) by UV–vis spectrophotometer (Analytik Jena, Specord S600, Germany). TEM measurements were carried out with a FEI Tecnai G2 12 by working voltage of 120 kV. The water contact angle of the film was measured by the dripping method over time, a 300 μL of ultra-pure water droplets were placed on the surface of the film, and the water droplets were recorded with a video camera over time, and the contours of the water droplets were calculated.

2.3.1. Water vapor transmission rate

The water vapor transmission rate of the edible multifunctional nanocomposite film adopts the American Society for Testing and Materials (ASTM) method E96-90 [26,27], which was evaluated after improvement. The sample was cut into a circle with a diameter of 20 mm, sealed on the mouth of a 12 mm-diameter glass bottle (clamp material is rubber band) containing 10 mL of deionized water, and then placed in an incubator at 34 °C and RH = 47 ± 2%. The weight of the bottle was recorded every 1 h for a until the plot should become linear (weight loss versus time). The water vapor transmission rate used the following formula to calculate:

$$WVTR\ (\mathrm{g\ mm\ m^{-2}\ day^{-1}}) = \frac{w \cdot d}{A \cdot t} \cdot 24 \tag{1}$$

where w is the weight loss (g) during time t (h), A is the effective area of the sample (m^2), and d is the thickness of the film (mm).

2.3.2. Oxygen permeability

Oxygen permeability of the edible multifunctional nanocomposite film was tested by the American Society for Testing and Materials (ASTM) method F3136-15 [28]. It was tested with professional oxygen permeability equipment (MOCON 221MD, United States). The sample was cut into a square of 120 mm × 120 mm, and then the thickness of the film was measured. The test was performed at room temperature. It is noteworthy that high purity oxygen was on one side of the film and 98% high purity nitrogen and 2% hydrogen were on the other side. Data was performed per 30 min for total of 11 times. And perform unit conversion based on the following formula:

$$OTR\ \left(\mathrm{cm^3\ m^{-2}\ day^{-1}}\right) = \frac{V}{A\cdot t} \tag{2}$$

$$OP\ \left(\mathrm{cm^3\ \mu m\ m^{-2}\ day^{-1}\ kPa^{-1}}\right) = \frac{OTR\cdot d}{P} \tag{3}$$

where OTR is the oxygen transmission rate, v is the oxygen transmission rate, t is the experimental test time, A is the effective area of the sample (m^2), d is the thickness of the film (μm), P is the oxygen partial pressure difference and OP is the oxygen permeability.

2.3.3. Cellular toxicity

The extract solution of the edible multifunctional nanocomposite film was obtained by immersing the materials in serum cell culture medium for 24 h at 37 °C, after 24 h, the extract was obtained and stored at 4 °C. The human skin fibroblasts (HS668) were applied for this test. These cells were cultured in RPMI 1640 medium at 37 °C. The cells were incubated in 96-well cell plates with 5000 cells. After the cells adhered to the wall sufficiently, the medium was replaced by different solutions (0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 3.0 mg/L, 4.0 mg/L, 5.0 mg/L) for 24 h. The media in the well plates were removed, and medium containing CCK-8 reagent (10% v/v) was added to each well and incubated for 4 h at 37 °C. The OD value was tested at 450 nm and the relative growth rate (RGR) was calculated by using the following formula [29]:

$$RGR(\%) = \frac{OD_{sample}}{OD_{blank}} \times 100\% \tag{4}$$

2.3.4. Mechanical properties

The mechanical performance test was carried out on the universal stretching machine (INSTRON 3369, America), which was carried out at 25 ± 1 °C. The films were cut into 10 mm × 100 mm, and the two ends were clamped on the clamp of the universal stretching machine, which were spread at a speed of 5 mm/min until the sample breaks. Tensile strength (MPa) was calculated using Eq. (5), elongation break was calculated using Eq. (6) [30].

$$Tensile\ Strength\ (\mathrm{MPa}) = \frac{F}{x\cdot w} \tag{5}$$

where F is the maximum tensile strength (N), x is the thickness of the film (mm), and w is the width of the film (mm).

$$Elongation\ Break(\%) = \frac{L}{L_0}\cdot 100\% \tag{6}$$

where L is the displacement length at the break (mm), and L_0 is the film's initial length (mm).

2.3.5. Antibacterial activity

The antibacterial activity test was carried out by the filter paper method, and the indicator bacteria used were *Escherichia coli* and *Staphylococcus aureus* in the ultra-clean workbench [31]. First, the sterile agar solution was poured into a sterile culture dish and waited for the agar to solidification. The *Escherichia coli* (1×10^7 cfu/mL) and *Staphylococcus aureus* (1×10^7 cfu/mL) were inoculated on different sterile agar plates. The filter paper was cut through a hole punch and then sterilized and soaked in the FSG film (control group) and solution edible multifunctional nanocomposite film solution (experiment group), then it was put on the agar plates with bacteria. Finally, the culture dishes were sealed and in a placed at 37 °C for 18 h to observe the antibacterial effect.

2.3.6. Contact angle for fruit peer (longan and banana) and wetting properties

The contact angle of the film-forming solution on the banana and longan skin were measured by the drop method (JY-PHb, Chengde, China), this measurement was carried out at room temperature (26 ± 1 °C), We using water as a control and film-forming (FSG, FSG&$CaCO_3$ and FSG&$CaCO_3$&Chitosan) solution as a treatment.

The longan and banana surface wettability was determined. The surface tension (γ_L) of the wetting solutions (measured by JYW-200, Chengde, China) and contact angle (θ) between the solutions and solid surface (longan and banana peel). Calculate the work of adhesion (W_a), work of cohesion (W_c) and spreading coefficient (W_s) following formulas (7–9).

$$W_a = \gamma_L\cdot(1 + cos\theta) \tag{7}$$

$$W_c = 2\cdot\gamma_L \tag{8}$$

$$W_s = \gamma_L\cdot(cos\theta - 1) \tag{9}$$

2.4. Application

2.4.1. Application of films on longan

The fruits of the longan were bought from the Longan Technology Demonstration Field (Nan'an, Fujian, China). The fruits were selected on the basis to the uniform size, similar color, no pests and diseases. The selected fruits were separated into three groups, the control group was dipped in pure water for 30 min, other two groups were dipped in FSG&$CaCO_3$ and FSG&$CaCO_3$&Chiosan solutions for 30 min, respectively. After all the longans were then taken out and dried, they were packed in 0.015 mm polyethylene bag (30 longans per bag), stored at (28 ± 1) °C and 90% humidity. These samples (peels and pulps) were recorded every other day to observe the changes [32,33].

2.4.2. Longan weight loss percentage

The weight of longan in the polyethylene bag was recorded every day for a total of 8 days. The weight loss percentage was calculated by using the following formula [34]:

$$Weight\ Loss\ Percentage(\%) = \left(1 - \frac{w_t}{w_0}\right)\cdot 100\% \tag{10}$$

where w_t represents the weight of the fruit at time t, and w_0 represents the initial weight of the fruit.

2.4.3. Application of films on banana

The banana was selected on the basis to the uniform size, similar color, no pests and diseases. The selected banana was separated into three groups, the control group was dipped in pure water for 30 min, other two groups were dipped in FSG&$CaCO_3$ and FSG&$CaCO_3$&Chiosan solutions for 30 min, respectively. After all the banana were then taken out and dried, they were packed in 0.015 mm polyethylene bag (3 bananas per bag), stored at (28 ± 1) °C and 90% humidity.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed on the data, and statistical analysis was performed using the SPSS program to determine the significance of each value ($P < 0.05$). The graphs were completed by using Origin2018 software.

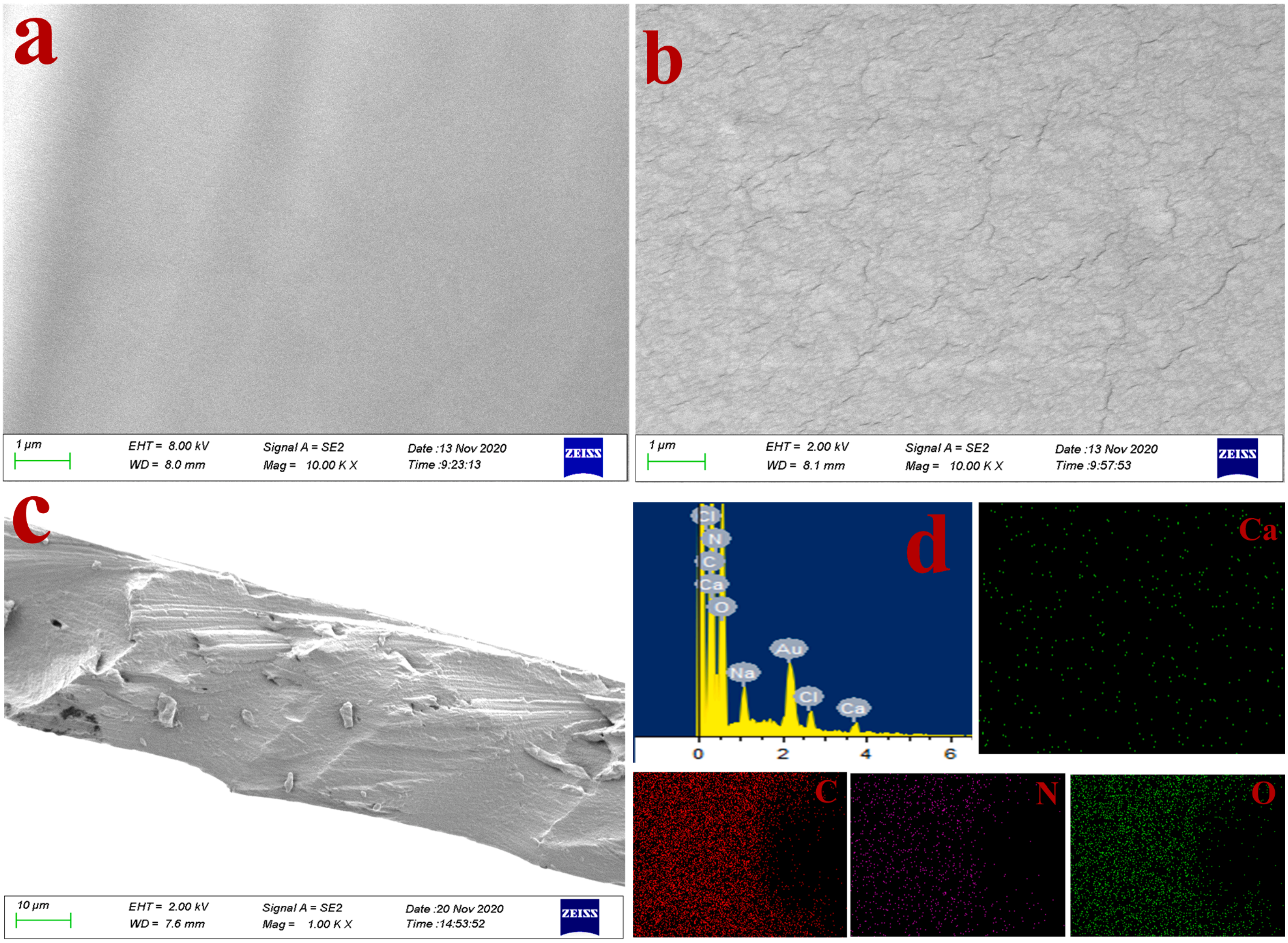

Fig. 1. a) SEM image (Mag = 10.00K×) of the fish gelatin film. b) SEM image (Mag = 10.00K×) of the edible multifunctional nanocomposite film. c) SEM image (Mag = 1.00K×) of a cross-section of edible multifunctional nanocomposite film. d) EDX spectra as element mapping of Ca, C, N, O for the edible multifunctional nanocomposite film.

3. Results and discussion

3.1. Scanning electron microscopy (SEM)

Surface morphology and texture plays a key role in determining the performance of as-prepared multifunctional nanocomposite film. Thus, SEM characterization experiments were carried out to examine the microstructure of the film [35]. Fig. 1a is the SEM image of the film, which is made up of fish scale gelatin and glycerin. One can see that its surface is smooth and compact. With the involvement of nano-$CaCO_3$ (proved by following XRD) and chitosan, the nanocomposite film shows a totally different surface morphology, as shown in Fig. 1b. It is a little rougher than the film in Fig. 1a, but still looks like regular and uniform. This can be proved by EDX, in which the elements of Ca, C, N, O are uniformly distributed. Furthermore, the cross-sectional SEM of the nanocomposite with a width ca. 40 μm is shown in Fig. 1c, which also has a smooth and dense structure. In contrast, the cross-sectional SEM of pure fish scale film indicates a porous and loose structure (Fig. S1, Supporting information). SEM observation shows that our film is well fabricated and there are no bubbles or other defects occurred during the casting process. On the other hand, it also indicates that the added chitosan and nano-$CaCO_3$ are well dispersed in the fish scale gelatin matrix, which can be attributed to the similar functional groups caused among them. It is well-known that chitosan mainly contains -NH_2 and -OH groups, in contrast, gelatin mainly amide groups. However, adding nano-$CaCO_3$ into the mixed solution might partly block the hydrogen bond interaction and destroy the orderly arrangement between them, leading to the formation of new ionic and covalent bonds. This might be the reason that the surface of nanocomposite is a little rougher than the film without the addition of nano-$CaCO_3$. Despite all this, nano-$CaCO_3$ will play key roles in determining the other performances of the nanocomposite, which will be explained in the following experiments.

3.2. Fourier-transform infrared spectroscopy (FTIR)

FT-IR spectra of the FSG film and the edible multifunctional nanocomposite film were shown in Fig. 2a. One can find that the spectra of two films have similar bands between 3300 cm^{-1} and 1396 cm^{-1}. Furthermore, it is evident that the peak at 3270 cm^{-1} corresponds to the N—H stretching vibration. The C—H anti-symmetric stretching vibration of the amide B band is located at 2938 cm^{-1}. The peaks at 1635 cm^{-1} and 1552 cm^{-1} are attributable to C═O stretching vibration and N—H bending vibration, respectively. It is the absorption peak of amide III at 1396 cm^{-1} [36]. The characteristic absorption band at 1152 cm^{-1} belongs to the NH_2 oscillation vibration of the primary amide in fish scale gelatin. In contrast, the addition of chitosan and $CaCO_3$ nanoparticles makes the edible multifunctional nanocomposite film give rise to several extra peaks, which is different with the fish scale gelatin film. The two peaks at 1105 cm^{-1} and 1030 cm^{-1} correspond to the characteristic peaks of -OH and -C-O-C- in chitosan [37]. The peak at 852 cm^{-1}

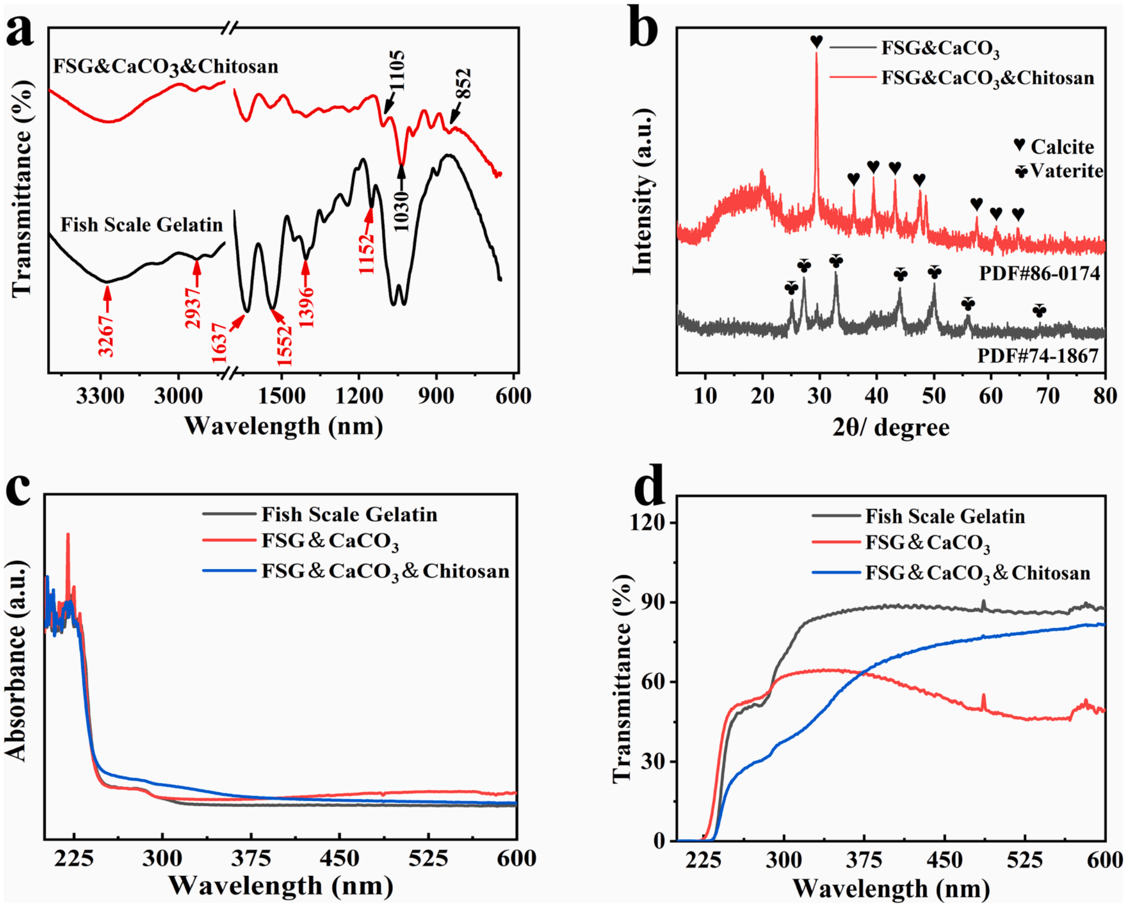

Fig. 2. a) FTIR spectra of natural FSG film and the edible multifunctional nanocomposite film. b) The XRD patterns of the obtained nano-$CaCO_3$ crystals. c) The UV spectrum of the natural fish scale gelatin film, FSG&$CaCO_3$ film, and the edible multifunctional nanocomposite film. d) The light transmittance of fish scale gelatin film, FSG&$CaCO_3$ film, and the edible multifunctional nanocomposite film.

and 706 cm^{-1} (Fig. S2, Supporting information) corresponds to the CO2–3 outer surface curvature [38]. Additionally, due to the Ca cross-linking, the strength of absorption rate in some peaks (such as C—H, N—H, etc.) for the edible multifunctional film is significantly reduced. Through FTIR analysis, the edible multifunctional nanocomposite film has many new peaks and strength changes, which are the results of cross-linking of fish scale gelatin, chitosan and $CaCO_3$ together.

3.3. X-ray diffraction (XRD)

Considering the possible involvement of inorganic nanoparticles in the system, XRD experiments are carried out to analyze the phase and purity in the fish scale gelatin film and the edible multifunctional nanocomposite film. As shown in Fig. 2b, the diffraction peak in fish scale gelatin film appearing in 24.9°, 27.0°, 32.7°, 43.8°, 49.9°, 55.7°, 68.6°, corresponding to the faces of (110), (111), (112), (200), (114), (222), (224), matches well with the vaterite (PDF#74-1867). Interestingly, the diffraction peaks in edible multifunctional nanocomposite film appearing in 29.4°, 35.9°, 39.4°, 43.2°, 47.1°, 57.4°, 60.7°, 64.7°, corresponding to the faces of (104), (110), (113), (202), (024), (122), (214), (300), match well with calcite (PDF#86-0174). This indicates that under the effect of acetic acid, the crystal forms of $CaCO_3$ nanoparticles have changed, from unstable state to stable one, Because vaterite is a relatively unstable phase of $CaCO_3$ [39]. In addition, no additional diffraction peaks are observed in the XRD pattern, revealing that the resulting $CaCO_3$ is a pure phase [40].

3.4. UV–vis spectroscopy and light transmittance

Fruits may deteriorate due to oxidation reactions caused by ultraviolet radiation during storage. Adequate UV protection can effectively slow down fruit rot degree. For the purpose of understanding the UV blocking function of the film, we tested UV–vis spectroscopy of the film. As shown in Fig. 2c, the natural fish scale gelatin, FSG&$CaCO_3$, and edible multifunctional nanocomposite film all have high UV absorption peaks at 200–250 nm. This is because that there are common aromatic chromophores such as peptide bonds (200–250 nm), tyrosine, and phenylalanine in fish scale gelatin [41]. Although the ultraviolet absorption peak at 250–300 nm is not apparent, the absorbance value of the edible multifunctional nanocomposite film is higher than that of fish scale gelatin and FSG&$CaCO_3$, which may be caused by the addition of chitosan [42]. In addition to UV protection, other optical properties of the edible multifunctional nanocomposite film wrapped on the fruit's surface can directly affect consumers' purchase intentions. Higher transparency can show the color of the fruit itself, as shown in Fig. 2c, the absorption peak at 300–600 nm for nanocomposite film is very low (close to 0), indicating the vis-light can almost completely penetrate the film. For packaging applications, visible light transparency is an important factor for edible film, especially the film is coated on the surface of fruit, so a good visible light transmittance can improve the fruit aesthetic property. Thus, we tested the light transmittance of the film at 200–600 nm. As shown in Fig. 2d, the light transmittance of FSG film at 390–600 nm is near to 90%, the light transmittance of FSG&$CaCO_3$ is ca. 51.3%, the light transmittance of nanocomposite film is ca. 76.4%. Obviously, the pure-fish scale made film has the highest

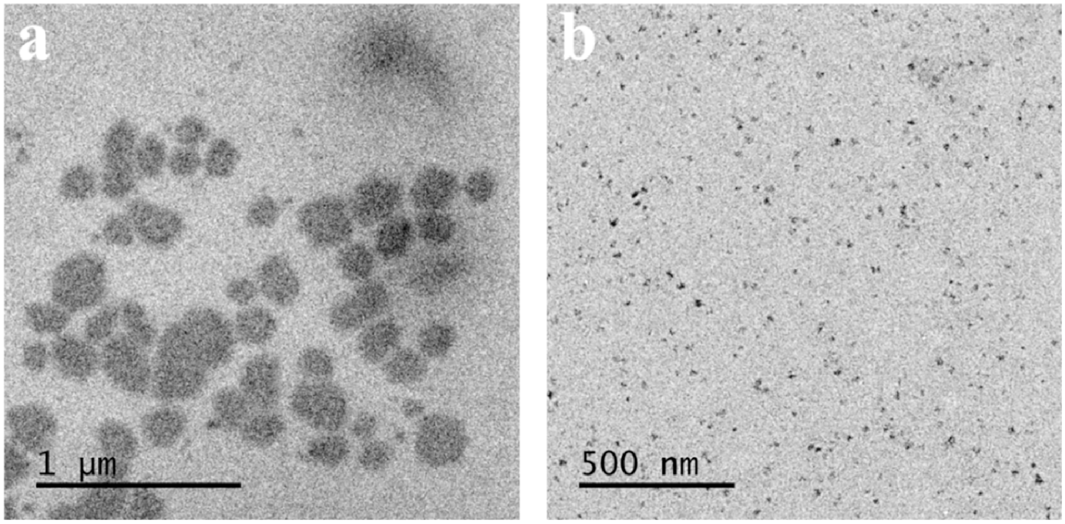

Fig. 3. a) TEM image of the FSG&$CaCO_3$ film solution. b) TEM image of the FSG&$CaCO_3$&Chitosan film solution.

Table 1
The mechanical properties comparison with other different packaging materials [34].

Materials[a]	Tensile strength [MPa]	Elongation [%]
Pectin	2.5–15	3.0–16.5
Starch	1.6–22.3	3.0–59.0
Protein isolate	0.2–10.0	12–468
Chitosan	7–42	12–72
Carnauba wax	1.6	0.2
FSG film	**1.8–2.3**	**199.8–225.7**
FSG&Chitosan film	**3.7–4.8**	**53.2–68.4**
FSG&$CaCO_3$&Chitosan film	**7.2–8.4**	**33.1–34.7**

[a] The properties have a broad range based on modifiers used.

value because it only contains gelatin. The nanocomposite film containing more matters (especially the addition of $CaCO_3$ nanoparticles) usually scatters more lights, resulting in the loss of transparency. Compared with the nanocomposite film, the FSG&$CaCO_3$ film shows a lower transmittance value. This is because that the size of $CaCO_3$ nanoparticles is totally different in two kinds of films. To further identify the difference, TEM was carried out in the following experiment.

3.5. Transmission electron microscope (TEM)

As shown in Fig. 3a, the $CaCO_3$ nanoparticles formed through in-situ way had the average size of 180 nm and were well dispersed. However, when chitosan was added into the in-situ prepared $CaCO_3$ nanoparticles solution, the smaller nanoparticles with average size of 20 nm were observed, as shown in Fig. 3b. Since the chitosan solution is acid (dissolved by acetic acid), it can effectively reduce the size of $CaCO_3$ nanoparticles into the certain scale. This is also the reason that the transmittance and optical clarity of the FSG&$CaCO_3$&Chitosan film are higher than the FSG&$CaCO_3$ film due to the small size effect of $CaCO_3$ nanoparticles.

3.6. Mechanical properties

The superior mechanical properties of the films guarantee the fruits to have a good protective behavior in the different postharvest processes (processing, storage, transportation and sales). After repeated bending the film for many times, it will not break down, which is one of the requirements for excellent packaging film. Thus, the mechanical properties of FSG film, FSG&Chitosan film, FSG&$CaCO_3$&Chitosan film were tested and compared with other reported films, as shown in Table 1. From it, one can see that the value of elongation break (EB) of FSG film is 199.8–225.7%, which is the biggest among them. But, its tensile strength (TS) is only 2.1 MPa. It is well known that the glycerin is usually used as a plasticizer to increase the film-forming ability. When it is added into gelatin solution, the intermolecular force of the protein chains is reduced, leading to the increase of the fluidity and flexibility of protein chains. Therefore, the FSG film shows good stretchable and flexible performance, but low tensile strength. That is to say that a small amount of force can deform the film, which will lose the protective effect for fruits. Compared with FSG film, the TS of FSG&Chitosan film is higher (reaching 4.3 MPa), it may be that the plasticizer has improved the compatibility of fish scale gelatin and chitosan, at the same time, fish scale gelatin and chitosan have similar functional groups, so that their molecules are more closely cross-linked. On the other hand, the decrease of TS is usually accompanied by the increase of EB, which determines the toughness of the composite film. Interestingly, the edible multifunctional nanocomposite film with addition of nano-$CaCO_3$ has a higher TS (7.8 MPa) and a lower EB (33.9%), these values are equivalent to those of most films (such as pectin, starch, protein isolate, chitosan, carnauba wax). Obviously, the involvement of nano-$CaCO_3$ increases the tensile strength, which might be attributed to the formation of new chemical bonds among them (gelatin, chitosan, glycerin, as well as $CaCO_3$), making the components more closely connected and forming a denser structure (as observed in Fig. 1c), enabling the edible film withstand emergency [43].

3.7. Cellular toxicity

As an edible film, edibility and safety is primary. So, we evaluate the cell toxicity of the nanocomposite film through in vitro studies on human skin fibroblasts [44]. A series of different concentrations films (0.5 to 5 mg/L) were incubated the cells for 24 h. It can be seen from Fig. 4a, there is no significant change in the survival of human epithelial cells. For the testing groups, more than 95% of the human skin fibroblasts survived, which indicates that the edible multifunctional nanocomposite film is not cytotoxic and has no food safety concerns, when were used for fruit coatings. This result can also be expected, because that the original materials used (fish scale gelatin, chitosan, glycerin, nano-$CaCO_3$) in the experiments are all naturally-existed in nature, and their ingredients are safe and efficient to human health.

3.8. Water vapor transmission rate

To control the moisture transmission in the surrounding

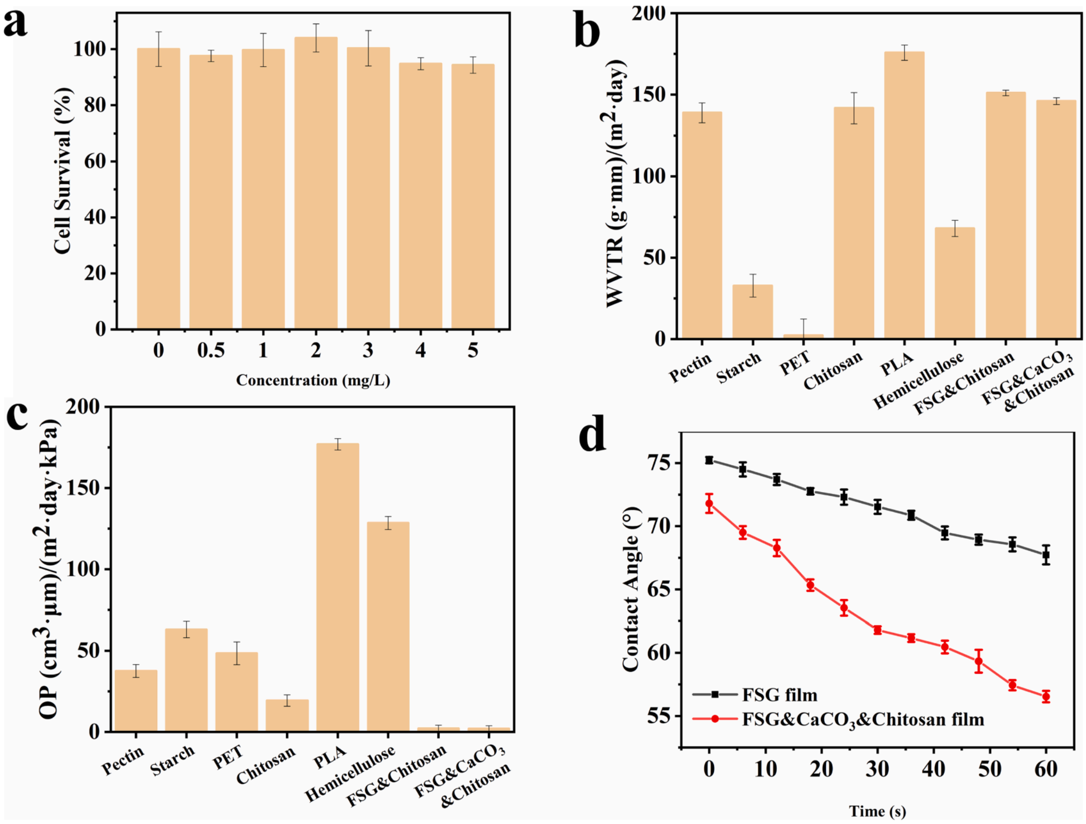

Fig. 4. a) Hs68 cell survival percentage after incubation with different concentrations of the edible multifunctional nanocomposite film, b) water vapor transmission rate of the edible multifunctional nanocomposite film. c) The oxygen transmission rate of the edible multifunctional nanocomposite film. d) Change of the water contact angle with time.

environment, the film needs to possess an appropriate ability to prevent water vapor loss on the surface of fruits [45]. As shown in Fig. 4b, we measured the water vapor transmission rate of the edible multifunctional nanocomposite film with a 40 μm thick at the condition of 34 °C and RH = 47 ± 2%. The results demonstrate that the water vapor transmission rate of the nanocomposite film is ≈146.04 g mm m^{-2} day^{-1}, respectively, which is lower than PLA (175.79 g mm m^{-2} day^{-1}), close to the values of Pectin (138.91 g mm m^{-2} day^{-1}) and Chitosan (141.79 g mm m^{-2} day^{-1}), but higher than starch (32.85 g mm m^{-2} day^{-1}), PET (2.31 g mm m^{-2} day^{-1}), hemicellulose (68.01 g mm m^{-2} day^{-1}) and FSG&$CaCO_3$ film (151.07 g mm m^{-2} day^{-1}). Although the value of water vapor transmission rate of our film is not the best one, the low-cost, biodegradability, good mechanical performance as well as UV protection ability enable it become the optimum among them. However, as an ideal edible film, it should have excellent function to hinder the loss of water vapor. Considering the strong water-vapor blocking ability of starch and hemicellulose, the future efforts will focus on improving the hydrophobicity and reducing the water vapor transmission rate of our material with part addition of these naturally bio-macromolecules. Related work is in progress in our lab.

3.9. Oxygen permeability

Another critical parameter that affects fruit preservation is oxygen. Oxygen is involved in fruit respiration and oxidative browning on the surface of the fruit. As shown in Fig. 4c, the OP value of oxygen barrier capacity of the edible multifunctional nanocomposite film and FSG&$CaCO_3$ film are ≈0.39 and 0.37 cm^3 μm m^{-2} day^{-1} kPa^{-1}, respectively, which is much lower than those reported ones, such as pectin, starch, PET, chitosan, PLA, hemicellulose [34]. This also explains that the fruits treated with the edible multifunctional nanocomposite film can extend the shelf life, because the lower oxygen content between the fruit peel and the protective film can reduce the cell respiration rate and delay the fruit ripening process. Generally speaking, compared with pure chitosan, chitosan-gelatin blend film has better oxygen barrier properties, because gelatin and chitosan will crosslink through intermolecular bonding (such as electrostatic attraction and hydrogen bonding), make the polymer matrix structure denser. In addition, according to the previous report, when oxygen passes through the chitosan-based film containing large number of NH+ 3, O_2 will associate with NH+ 3, which changes the solubility of oxygen, resulting in the robust blocking ability of O_2 [46]. The edible nanocomposite film with strong oxygen barrier character will further keep the fruits from oxidation.

3.10. Surface wettability of film

To evaluate the durability of the edible coating on the surface of the fruit, as well as the wettability, hydrophilicity and hydrophobicity of the film, we tested the contact angle of the water droplets on the films. When the value of the contact angle is less than 90°, the film surface is hydrophilic; on the contrary, when the value of the contact angle is greater than 90°, the film surface is hydrophobic. As shown in Fig. 4d, the contact angle of pure fish scale gelatin film decreased from 75.2° to 67.7° within 1 min, while the contact angle of edible multifunctional nanocomposite film decreased from 71.8° to 56.5° within 1 min. Obviously, both kinds of films are hydrophilic, in which lots of hydrophilic groups are contained, such as amide, carboxyl, hydroxyl as well as amino groups, as proved by FTIR. It is worth noting that the fish scale gelatin contains amide groups, meanwhile, the edible multifunctional nanocomposite film includes an abundance of extra carboxyl, amino, as well as hydroxyl groups, making the edible multifunctional

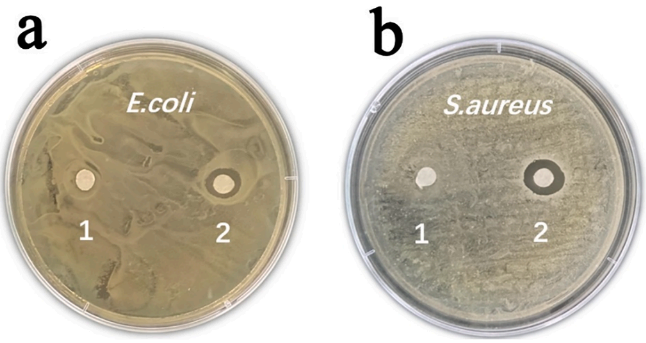

Fig. 5. a) Antibacterial activity of edible multifunctional nanocomposite film against *Escherichia coli*, b) *Staphylococcus aureus*. 1 group: film without chitosan; 2 group: film with addition of chitosan.

nanocomposite film more hydrophilic than the fish gelatin film. The hydrophilic character of nanocomposite film enables it to be easily washed out before consumers eat the fruits.

3.11. Antibacterial activity

Fresh fruits often rot and deteriorate due to the diseases infected by microorganisms, which seriously affect their edible value and shelf life. Some Microorganisms can also produce toxins, which are carcinogenic or other toxic, causing huge economic losses and human health problems. Here, we used *Escherichia coli* and *Staphylococcus aureus* to evaluate the antibacterial activity of the edible nanocomposite film. As shown in Fig. 5. No inhibition zones appeared in the groups without chitosan, indicating no bacterial effects. In contrast, two evident inhibition zones in the groups with chitosan are observed in two separate plates, indicating that the edible multifunctional nanocomposite film has antibacterial effects on both bacteria. The satisfying antibacterial activity of edible nanocomposite film endow the fruit with strong protection capability, which can resist microorganisms attack, slow down the spoilage, and even reduce the outbreak of human foodborne diseases. It should be noted that the antibacterial ability of our film is attributed to the chitosan, which has a broad spectrum of antimicrobial effects against Gram-negative bacteria, Gram-positive bacteria, as well as fungi.

3.12. Wettability of longan and banana with film-forming solutions

The contact angle of droplets of film solutions on the longan and banana skin was lower than that of water (Fig. 6a and b). This suggests a high interaction of the film solutions with the longan skin and banana skin surface. The contact angles of longan and banana decreased about 34% and 16% (compared with water) by adding nano-CaCO3 to FSG&Chitosan, respectively. Therefore, further increasing the surface wettability by the active coating.

The surface wettability is an important factor in determining adhesion strength. Lower equilibrium contact angle correspond to increased adhesion strength [47]. As shown in Fig. 6c and d, the adhesion strength, cohesive energy, and spreading coefficients of the coating solutions (FSG, FSG&$CaCO_3$ and FSG&$CaCO_3$&Chitosan) on the longan and

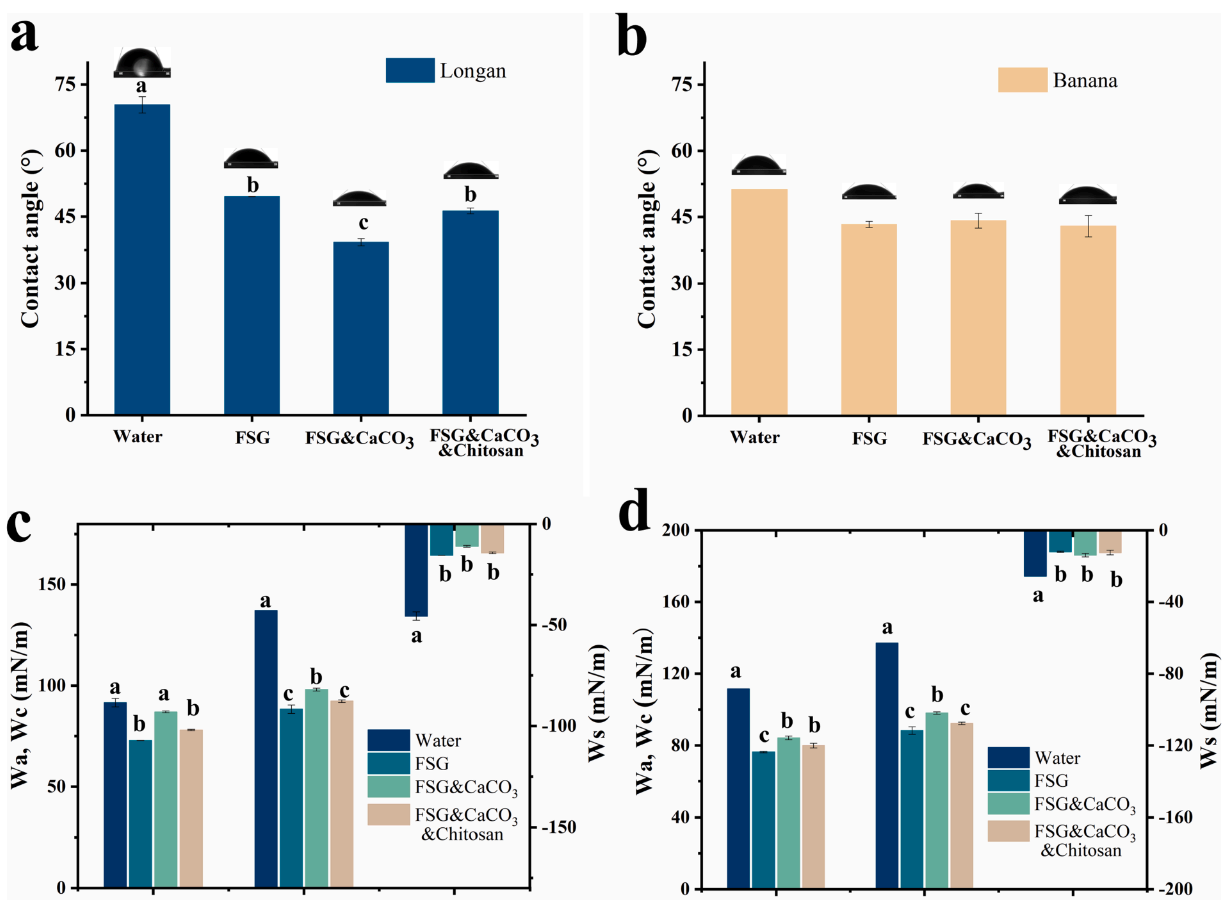

Fig. 6. a) and b) show average contact angle of water, FSG solution, FSG&$CaCO_3$ solution and FSG&$CaCO_3$&Chitosan solution to longan skin and banana skin, respectively. c) and d) show adhesion (W_a), cohesion (W_c) and spreading (W_s) of coating solutions on longan skin and banana skin, respectively. Different letters denote significant differences ($P < 0.05$).

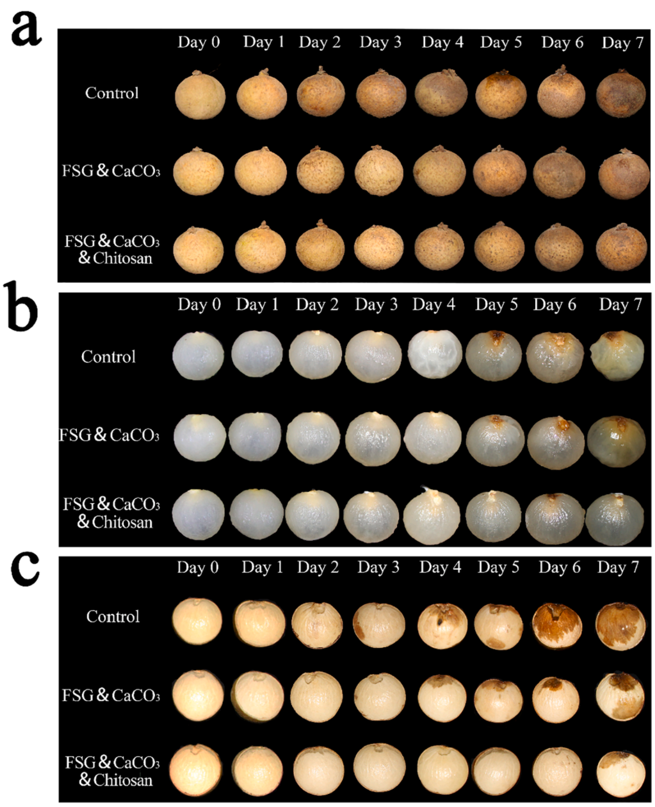

Fig. 7. The effects of different material coatings on fruit ripeness. a) Time-lapse photographs of coated with pure water (control) and FSG&$CaCO_3$ solution and FSG&$CaCO_3$&Chitosan solution longan fruit. b) Time-lapse photographs the inner appearance of pure water (control) and FSG&$CaCO_3$ solution and FSG&$CaCO_3$&Chitosan solution treated longan fruit. c) Time-lapse photographs of the fruit inner peel, which treated by pure water, FSG&$CaCO_3$ solution and FSG&$CaCO_3$&chitosan solution.

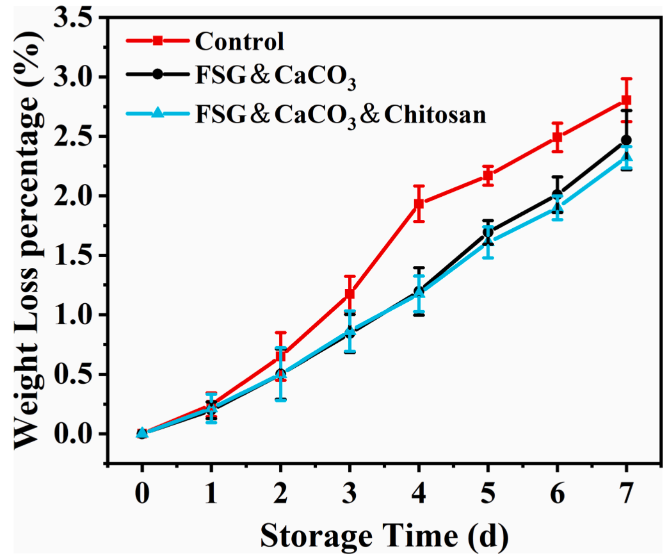

Fig. 8. The relationship between longan weight loss percent and different storage time for three different treatments.

banana (respectively) surface in comparison with water. Wettability of coating solution to solid surface can be attributed to the equilibrium mechanism between adhesive force and adhesive force [48]. The adhesion force indicates the spreading ability of the liquid, and the cohesion force its contraction tendency [49]. As shown in Fig. 6c and d, except that the adhesion coefficient of FSG&CaCO3 in longan and water has no significant difference, the adhesion coefficients of the others (FSG, FSG&$CaCO_3$&Chitosan, and FSG&$CaCO_3$ in bananas) have significant differences with water. At the same time, the cohesion coefficients of coating solutions (FSG, FSG&$CaCO_3$, FSG&$CaCO_3$&Chitosan) were lower than that of water. These results show the high wettability of longan and banana surface for the containing FSG element solutions.

The spreading coefficient is one of the most important indicators evaluating the surface ability of a solution coating, the spreading coefficient values closer to zero indicate better surface wetting by the tested liquid [47]. Notably, the containing FSG element solutions exhibited a higher spreading coefficient, and it can reduce the surface tension to improve the adhesion on the longan and banana surface. At the same time, the morphological properties of the coatings (FSG, FSG&$CaCO_3$ and FSG&$CaCO_3$&Chitosan) were characterized by optical microscopy. Fig. S3 showed that the sequential dipping of banana and longan in different coating solutions enabled the formation on the surface of a continuous and homogenous. This layer appeared as compact and did not show any visible separation in different phases or pore formation.

3.13. *Application of films on longan preservation*

To examine the effect of the edible nanocomposite films on the shelf life of longan fruits, FSG&$CaCO_3$ solution, FSG&$CaCO_3$&Chitosan as well as pure water (as control) solutions were compared. Herein, we directly dipped the longan fruits into three different solutions (in aqueous medium) by adopting the dip coating method, which is usually used for the treatment of vegetables, fruits and meat. After short immersing time, they were taken out from the container and dried naturally in air, leading to the formation of a layer of membrane over the surface of targets [50]. The results were shown in Fig. 7. As can be seen in Fig. 7a, with the increasing storage time, the outside peels of longan fruit in three different treatment groups all showed rottenness in various degrees. The control group without film protection had the most awful appearances on the 7th day, revealing the shortest storage time. Notably, the FSG&$CaCO_3$&Chitosan group showed the satisfying appearances among them at the same time, revealing the longest storage time. To further observe the inside changes of longan in detail, Longan pulp and peel were separated from the fruit. As shown in Fig. 7b, the fresh edible pulp showed us a translucent-white and fleshy aril at the beginning. However, the control group and the FSG&$CaCO_3$ group began to give rise to "autolysis" phenomenon on the 4th day. In contrast, the FSG&$CaCO_3$&Chitosan group had no obvious "autolysis" until the 7th day. The same results are also observed in the inside of longan peels, as shown in Fig. 7c. Furthermore, the chromaticity values of the longan peels were measured (Fig. S4), which is consistent with the result of visual study. In addition, banana fresh-keeping experiments (see Figs. S5, S6) were also carried out to identify the universality of the as-prepared film, which showed the same satisfying results and significant advantages.

3.14. *Fruit weight loss percentage (longan)*

Due to the transpiration and respiration of the fruit, it is easy to lose water and weight followed by picking and the extension of storage time, which makes the fruit shrivel and become smaller, leading to the deterioration of exterior quality. Therefore, the water loss rate can be used as an important index to evaluate the freshness of fruits. Longan, a kind of fruit widely planted in tropical area, is chosen as a model to assess the preserving water effect. Fig. 8 shows the changes of water loss rate of longan with the storage time for three different treatment conditions (control group with water dipping, FSG&$CaCO_3$ group, FSG&$CaCO_3$&Chitosan group). It can be seen that the weight loss rates of control group, FSG&$CaCO_3$ group, FSG&$CaCO_3$&Chitosan group are 2.81%, 2.47%, 2.32% at the seventh day storage, respectively. Obviously, the

FSG&$CaCO_3$&Chitosan group has the lowest values, revealing the excellent water-blocking ability of the edible multifunctional nanocomposite film again.

4. Conclusions

In summary, a biodegradability, sustainability, multifunctional and flexible nanocomposite for fruits coating and packaging was successfully developed by using the waste fish scale, glycerin, chitosan, as well as nano-$CaCO_3$. The primary materials were derived from the most abundant biopolymers, which can powerfully solve the problems of white pollution and marine waste treatment (such as fish scale garbage). The as-prepared nanocomposite showed good structure and multifunctional characters, such as UV absorption, antimicrobial, oxygen barrier, non-toxicity and available tensile strength, etc. Importantly, the nanocomposite material was used for actual fruit preservation, which can effectively prolong the shelf life of longan and banana. Although the nanocomposite film has a weak water-resistance ability, this disadvantage will be overcome by addition of some hydrophobic materials in near future. It is suggested that fish scale combing with some natural biomacromolecules may be a promising "green and cheap" precursor to fabricate the environment-friendly edible multifunctional food-wrapping film.

CRediT authorship contribution statement

Bofei Fu: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing - original draft, Visualization. Shanshan Mei, Xianjie Su and Hongbin Chen: Formal analysis, Investigation. Junqiu Zhu: Validation. Zongping Zheng: Writing - review. Hetong Lin: Supervision. Congjie Dai: editing. Da-Peng Yang and Rafael Luque: Resources, Writing - review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2021.09.171.

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